Why honey is effective as a medicine

2. The scientific explanation of its effects

PETER MOLAN

The effectiveness of honey as a therapeutic agent has been unequivocally demonstrated in the literature reviewed in Part 1 of this article published in 1999, but the biochemical explanation of these effects is more hypothetical. However, a rational explanation can be seen when one looks at the scientific literature outside that on honey. Some of the components of honey are substances known to have physiological actions that would explain many of its therapeutic effects. In addition, research on honey has shown directly that it has physiological actions that would give therapeutic effects.

Therapeutic properties of honey

Antibacterial activity

The large volume of published literature from laboratory studies that has established that honey has significant antibacterial activity has been comprehensively reviewed[92,93]. Since then there have been many other studies reported[5,14,15,24,37,38,40,51,53,55,67,104,110,122,144,145].

But much of the published work establishing the sensitivity of bacteria to honey has unfortunately not taken into account the marked variation in potency of different honeys. However, some studies have used honeys with median levels of activity so that the sensitivity of various species of bacteria to typical honeys could be determined. In one of these studies[150] the non-peroxide antibacterial activity of a typical manuka (*Leptospermum scoparium)* honey was tested against seven major wound-infecting species of bacteria in comparison with a typical honey with activity due to hydrogen peroxide. The MIC (minimum inhibitory concentration) of honey was found to range from 1.8% to 10.8% (v/v), i.e. the honey had sufficient antibacterial potency to still be able to stop bacterial growth if diluted at least nine times, and up to 56 times for *Staphylococcus aureus,* the most common wound pathogen. In another study of the same honeys against 20 isolates of *Pseudomonas* from infected wounds[37], the mean MIC was found to be 6.9% (v/v) (range 5.5% to 8.7%) for the manuka honey and 7.1% (v/v) (range 5.8% to 9.0%) for the other honey. A similar study with a range of clinical isolates of *S. aureus"* found the MIC to be between 2% and 3% (v/v) for the manuka honey and 3% and 4% (v/v) for the other honey.

Note: this article reports information, but does not constitute medical advice on the usage of honey

There is also clinical evidence for the antibacterial activity of honey being sufficient to achieve a therapeutic effect. In a clinical trial of honey for the treatment of diarrhoea it was found that administering honey halved the duration of diarrhoea caused by bacterial infection[64]. There are also reports of infected wounds dressed with hone becoming sterile in 3-6 days[25,31], 7 days[49,50,108] or 7-10 days[17], and the advance of infection through tissues halted[50,70]. Also it has been reported that honey provides a protective barrier that prevents wounds from becoming infected[20,49,91,128,129], and thus protects patients in hospital from cross-infection[55]. The clinical significance of the antibacterial activity of honey can be seen in reports of honey being effective on wounds not responding to conventional therapy with antibiotics and anticeptics[47,49,66,74,101,141,413,152] and a wound infected with the antibiotic-resistant MRSA (*methicillin-resistant Staphylococcus aureus)*[48].

The antibacterial activity of honey is very important therapeutically, especially in situations where the body's immune response is insufficient to clear infection. Bacteria often produce protein-digesting enzymes, which can be very destructive to tissues[135] and can destroy the protein growth factors that are produced by the body to stimulate the regeneration of damaged tissues in the healing process[112]. Furthermore, some bacteria produce toxins that kill tissue cells[43]. Additional damage is often caused by bacteria carrying antigens that stimulate a prolonged inflammatory immune response which gives excessive production of free radicals that are very damaging to tissues[61] (as discussed below). Bacteria in wounds can also consume oxygen and thus make the level of oxygen available to the wound tissues drop to a point where tissue growth is impaired[123]. The consequences of bacterial infection, avoided by administering honey to clear infection, are: non-healing of wounds; increase in size of wounds and development of ulcers and abscesses; failure of skin grafts; inflammation, causing swelling and pain.

Because of the large variation in antibacterial activity of honey, not all honey is likely to have the same therapeutic effect. Physicians in past millennia were aware of this, at least from practical experience, and specified particular types of honey be used to treat particular ailments. Dioscorides *(c.* 50 AD) stated that a pale yellow honey from Attica was the best, being 'good for all rotten and hollow ulcers'[62]. Aristotle (384-322 BC), discussing differences in honeys, referred to pale honey being 'good as a salve for sore eyes and wounds'[16] . There is a similar awareness in present-day folk medicine: the strawberry tree (*Arbutus unedo)* honey of Sardinia is valued for its therapeutic properties[57] in India, lotus *(Nelumbium sceciosum)* honey is said to be a panacea for eye diseases[59]; honey from the Jirdin valley of Yemen is highly valued In Dubai for its therapeutic properties[1]; and manuka honey in New Zealand has a long-standing reputation for its antiseptic properties.

Boosting the immune system

As well as having a direct antibacterial action, honey may clear infection through stimulating the body's immune system to fight infection. It has been reported that honey stimulates B-lymphocytes and T-lymphocytes in cell culture to multiply, and activates neutrophils[2]. It has also been reported that honey stimulates monocytes in cell culture to release the cytokines TNF-a, 1 and IL-6, the cell 'messengers' that activate the many facets of the immune response to infection. In addition to its stimulation of these leucocytes, honey provides a supply of glucose which is essential for the 'respiratory burst' in macrophages that produces hydrogen peroxide, the dominant component of their bacteria-destroying activity[117].

Furthermore it provides substrates for glycolysis, which is the major mechanism for energy production in the macrophages, and thus allows them to function in damaged tissues and exudates where the oxygen supply is often poor[117]. The acidity of honey may also assist in the bacteria-destroying action of macrophages, as an acid pH inside the phagocytotic vacuole is involved in killing ingested bacteria[117].

Anti-inflammatory action

The anti-inflammatory properties of honey have been well established. It has been observed clinically that when honey is applied to wounds it visibly reduces inflammation[30,132,154]. It has also been observed to reduce oedema around wounds[46,49,50,131] and exudation from wounds,[30,49,50,70] both of which result from inflammation. Pain is another feature of inflammation, and honey has been observed to be soothing when applied to wounds[30,81,129,154,155]. A histological study of biopsy samples from wounds has also shown that there are fewer of the leucocytes associated with inflammation present in the wound tissues[132]. What is responsible for these observations is a direct anti-inflammatory effect, not a secondary effect resulting from the antibacterial action removing inflammation-causing bacteria: the anti-inflammatory effects of honey have been demonstrated in histological studies of wounds in animals where there was no Infection involved[30,52,63,77,105,113]. A direct demonstration of the anti-inflammatory properties of honey, where honey decreased the stiffness of inflamed wrist joints of guinea pigs, has also been reported[35].

The anti-inflammatory action of honey is potentially very important therapeutically, as the consequences of inflammation can be major. Although inflammation is a vital part of the normal response to infection or injury, when it is excessive or prolonged it can prevent healing or even cause further damage.Some of the 'messengers' pr -oduced by the leucocs involved in inflammation to regulate the activity of surrounding cells are prostaglandins which cause the painful symptoms of inflammation. Others cause blood vessels to dilate and the walls of the capillaries to open up, so plasma flows out to cause swelling in the surrounding tissues. The pressure building up from this restricts the flow of blood through the capillaries[32], thus starving the tissues of the oxygen and nutrients that are vital for the cells to fight infection and multiply to repair damage. The swelling also increases the distance for diffusion from the capillaries to the cells[126]. The opening up of capillaries also causes exudation of serum from wounds and exudation of serum into the gut in gut infections, both of which can lead to malnutrition if they continue for a pronged period. But the most serious consequence of excessive inflammation is the production of reactive oxygen species (free radicals) in the tissues[56]. These arise through a series of reactions that are initiated by the production of superoxide by certain leucocytes that are stimulated to do so as part of the inflammatory process[115]. Free radicals can be extremely damaging as they are very reactive and can break down the lipids, proteins and nucleic acids that are the essential components of the functioning of all cells[36], so their continued production can lead to localized erosion of body tissues. The anti-inflammatory action of honey has been found in a clinical trial to prevent partial-thickness burns from converting to full-thickness burns which would have needed plastic surgery[132], a characteristic of burns, where there is much inflammation.

The free radicals formed in inflammation are also involved in stimulating the activity of the

Fibroblasts[34], which is the basis of the body's repair process, normally triggered by the inflammation that follows injury. These are the cells which are responsible for producing the connective tissue, including the collagen fibres of scar tissue, and in situations where there is prolonged inflammation their over-stimulation can lead to 'proud flesh' and fibrosis, an excessive production of collagen fibres[100]. The reduction in keloids and scarring that is a feature of the dressing of wounds with honey[50,128,130], and the cosmetically good results obtained[47], are probably due to the antiinflammatory action of honey. Thus, there are significant benefits to be derived from therapeutic use of anti-inflammatory substances. However, the pharmaceutical ones have serious limitations: corticosteroids suppress tissue growth and suppress the immune response[27], and the non-steroidal anti-inflammatory drugs are harmful to cells, especially in the stomach[26]. But honey has an anti-inflammatory action free from adverse side effects (see below).

Antioxidant activity

Honey has been found to have a significant antioxidant content[60], measured as the capacity of honey to scavenge free radicals. The antioxidant activity of honey has also been demonstrated as inhibition of chemiluminescence in a xanthine-xanthine oxidase-luminol system that works via generation of superoxide radicals[12]. This antioxidant activity may be at least partly what is responsible for the anti-inflammatory action of honey, as oxygen free radicals are involved in various aspects of inflammation, such as further recruitment of leucocytes that initiate further inflammation[44,56]. (The application of antioxidants to burns has been shown to reduce inflammation[136]) But even if the antioxidants in honey do not directly suppress the inflammatory process they can be expected, by scavenging free radicals, to reduce the amount of damage that would otherwise have resulted from these.

As well as scavenging free radicals to neutralize them after they have been formed, honey has the potential to exert an antioxidant action by a completely different mechanism, inhibition of the formation of free radicals in the first place. The superoxide that is first formed in inflammation is relatively unreactive, and is converted to hydrogen peroxide which is much less reactive, but from this is generated the extremely reactive peroxide radical[39]. This formation of the oxidant peroxide radical is catalysed by metal ions such as iron and copper, and sequestering of these metal ions in complexes with organic molecules is an important antioxidant defence system[65]. Flavonoids and other polyphenols, common constituents of honey, will do this[42].

Stimulation of cell growth

It has been observed clinically that when honey is used as a wound dressing it gives rapid healing of wounds[20,21,30]. It has been reported by many clinicians that honey promotes the formation of clean healthy granulation tissue (the clusters of fibroblasts around new capillary beds that is the regenerating connective tissue)[17,25,31,46,49,50,55,74,132,143]. It has also been reported that honey hastens epithelialization of the wound (coverage with a new outer layer of skin)[49,50,70,130,132], making skin grafting unnecessary[31,50,70,91,132]. This growth-stimulating property of honey has been confirmed histologically in many studies of wounds in animals[20,30,63,85,113], as has a stimulation of the synthesis of collagen fibres[134] and other connective tissue components[133] and improvement of the strength of collagen[134]. It has also been observed histologically in studies of wounds in animals that honey stimulates the development of

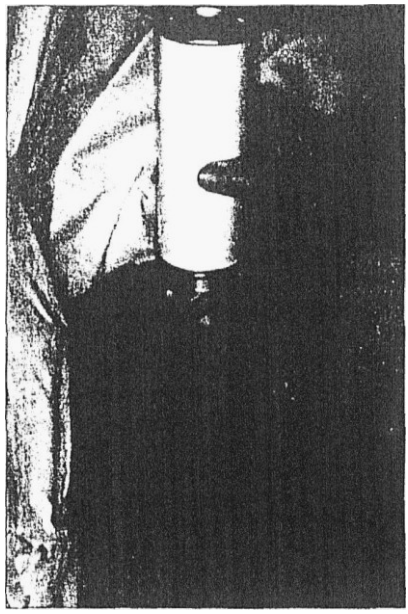

FIG. 1. Honey is harmless to tissues so can safely be used to fill deep abscesses. A prototype pressurized delivery system for doing this is illustrated.

new capillary beds[63,85], which is the rate-limiting factor in the formation of granulation tissue[123]. It is likely that it is the stimulation of cell growth by honey that is responsible for the 'kick-starting' of the healing process observed in chronic wounds which have remained non-healing for long periods[22,49,66,127,152].

Harmlessness of honey

The Hippocratic principle of doing no harm to the patient is particularly relevant to the selection of therapeutic agents, as most have untoward side effects. Antibiotics have numerous adverse side effects, and antiseptics are all toxic to some degree to the cells in body tissues and thus slow the healing process[137]. For example. in comparative trials on burns with silver sulfadiazine ointment, an antibacterial agent that is the standard treatment for burns in developed countries, it was found that significantly slower healing rates were achieved with this ointment than with honey[113,128,132] (Honey also gave a better control of infection than silver sulfadiazine ointment in these trials[128,132]). Honey has no adverse effects other than a stinging sensation experienced by some people when it is applied to open wounds[28,101,152]. A transient stinging sensation and redness of the eye soon after putting honey in the eye, but never enough to stop the treatment, was reported in the 102 cases in a trial of honey for ophthalmological use[54]. Over the thousands of years honey has been used on open wounds and in the eyes it has not gained any reputation for adverse effects, and this is borne out by histological examination of wound tissues that have been treated with honey[20,52,63,113]. In papers describing the application of honey to open wounds it is reported to be soothing[129], to relieve pain[129], be non-irritating[28,31,131], cause no pain on dressing[91], and give no secondary reactions[101]. Although allergy to antibiotics is fairly common, allergy to honey is rare[82]. It may be a reaction to either the pollen or the bee proteins in honey[18,71]. In reports of clinical studies where honey was applied to open wounds of a total of 134 patients it was stated that there were no allergic or adverse reac-tions[49,55,108,130,141].

Reference has been made to dehydration of tissues if too much honey is applied to an open wound, but it has been stated that the hydration of the tissues is easily restored by saline packs[11]. It has also been pointed out that although a piece of flesh removed from the body would dehydrate if exposed to a highly osmotic sugar solution, when blood is circulating in it this replaces from underneath any fluid withdrawn by osmosis[33].

There is a hypothetical risk of infection of wounds resulting from the application of honey, as honey sometimes contains viable spores of Clostridia[98]. However, in none of the more than 470 cases in the many reports published on the clinical usage of honey on open wounds was the honey that was used sterilized[94], yet there are no reports of any type of infection resulting from the application of honey to wounds. If spores germinated, any vegetative cells of Clostridia, being obligate anaerobes, would be unlikely to survive in the presence of the hydrogen peroxide that is generated in diluted honey. But any concern about risk of infection can be overcome by the use of honey that has been treated by gamma-irradiation, which kills Clostridia! spores in honey[97,111] without loss of any of the antibacterial activity[97].

There is also a risk of blood glucose levels in diabetics being raised by honey. There is also a hypothetical risk of blood glucose levels in diabetics being raised by honey, through glucose being absorbed from honey across the bed of large wounds, but in cases where this has been checked there has been no sign of this happening (I Betts, personal communication). Where honey is taken by mouth by diabetics for treatment of gastrointestinal infections the risk is greater, but research has shown that honey gives a lower peak of blood glucose than table sugar does because the absorption from the gut is slower[4,78,120]

Mechanisms of action of honey in therapeutic applications

Action of honey as a wound dressing

The report of G Winter in 1962[151], that wounds heal faster if kept moist than if a scab is allowed to form, was the start of what has become the standard modern approach to wound treatment, the prevention of drying out of a wound. The epithelial cells, which spread across the surface of a healing wound to restore the skin cover, need moist conditions to be able to grow. (When there is a dry scab on the surface of a wound the epithelial cells grow across in the moist area beneath it, and thus leave a pitted scar in the skin.) Also, the fibroblasts, functioning as a rudimentary form of muscle cells, need moist conditions to be able to contract and pull the margins of the wound together, A dressing of honey over a wound provides the moist conditions needed for these processes. The amount of free water in honey is very low, such as would be expected to dry out wound tissues. But the osmotic effect draws fluid out from below the honey dressing, and thus creates a layer of fluid that is a dilute solution of honey in plasma or lymph. A secondary benefit of this fluid layer is that there is no sticking of dressings to the surface of wounds when honey is used[28,91,129,132,147]. As well as giving painless dressing changes, this gives faster healing than with dry dressings because there is not the tearing away of the delicate newly re-grown tissues that adhere to the dressing when dry dressings (or even sometimes the modern moist wound healing dressing materials) are used. Combined with the stimulatory effects on tissue regeneration discussed above, this puts honey in the same category as the latest dressings produced by pharmaceutical technology, a bio-active moist wound dressing material.

One problem with using dressings that create a moist environment is that the moist conditions favour growth of bacteria, and for this reason some of the moister products in use are contra-indicated for use on infected wounds. But honey creates a moist environment in which bacterial growth is prevented by the antibacterial activity of the

honey. Furthermore, the antibacterial components of honey, unlike antibiotics, have a high solubility in water and thus can diffuse into the tissues. Honey has also been reported to give rapid deodorisation of offensively smelling wounds[49,50,70,91,108,128,129]. Whereas malodour is a common feature of the use of pharmaceutical moist dressings on wounds. It is probably more than just the antibacterial action of honey that is involved in removal of malodour: the high glucose levels that the honey provides would be used by the infecting bacteria in preference to amino acids[103] from the serum and dead cells, and thus would give rise to lactic acid instead of ammonia and the amines and sulphur compounds that are the cause of malodour in wounds.

Another advantage of having a moist wound-healing environment is that it allows the protein-digesting enzymes in the wound tissues to work and loosen any scab or pus and dead tissue. The alternative that often is necessary when this autolytic debridement is insufficient to achieve a clean wound bed is to use surgical debridement, as it is important to remove what would otherwise be a good culture medium for bacterial growth[68,126]. A more expensive option is to apply pharmaceutical enzyme preparations, or in some cases maggots that have 'been especially bred for this purpose. Honey has a very efficient debriding action, such that it is frequently remarked upon in papers reporting on the use of honey in wound treatment[21,28,31,46,49,50,55,70,74,101,128,129,131,143,146]. It has also been noted that dirt is removed with the bandage when honey is used as a dressing, leaving a clean wound[155]. The outflow of lymph caused by the osmotic effect of honey could be expected to help in this clearing of dirt from wounds.

Another beneficial effect that could be expected from the osmotic outflow of lymph caused by honey is increased nutrification of the tissues in healing wounds.

Whether caused by trauma or infection, at the site of tissue repair in wounds there are-often insufficient functioning blood vessels to supply the cells with the nutrients that they need to grow and multiply. The importance of this is demonstrated by the observation that wounds heal faster if a nutrient mixture is applied to them[80,102,124,142]. The drawing out of lymph would provide a constant flow of nutrients from the functioning blood vessels deeper down. Honey would in addition supply nutrients directly, not just readily metabolisable sugars but also a wide range of amino acids, vitamins and essential minerals[69,149]. The supply of glucose would be of particular importance to the epithelial cells which have to build up an internal store of carbohydrate to provide the energy they need to be able to migrate across the surface of the wound to restore skin cover [123].

The osmotic outflow of lymph induced by honey could also be expected to increase the oxygen supply to the tissues in healing wounds. Because of destruction of the local circulation there are insufficient functioning blood vessels around a wound to supply the cells with oxygen, thus growth of the cells repairing the wound is restricted[73]. Additional oxygenation of wound tissues is also likely to be induced by the acidity of honey, this being one of the two mechanisms proposed[86] to account for the finding that acidification of wounds increases the rate of healing[79,86]. The other mechanism proposed is the conversion of the toxic form of ammonia, NH_3 (produced in wounds by bacterial decomposition of protein), to the non-toxic ionic form, NH_4^+, that is the predominant form in an acidic environment[86]. As an acidulant for wounds, honey has the advantage of having a gentle action because the acidic component of honey, gluconic acid, exists mostly in the form of a neutral lactone that is in a slowly-converting equilibrium with the free acid form.

Action of honey in treating diarrhoea

The shortening of the duration of diarrhoea by administering honey in a clinical trial was attributed to the antibacterial activity of honey[64], which was in line with the finding that in the patients in this trial who had diarrhoea due to a viral infection there was no shortening of the duration by the honey treatment. (It was of significance that the duration of the viral diarrhoea was not increased by the antibacterial activity of honey, as commonly happens with other antibacterial therapy.) But it has also been suggested that the effectiveness of honey in treating diarrhoea may be due to it effecting repair of the intestinal mucosa (the lining of the intestines) damaged by the infection[88]. This suggested mode of action would be in line with the effect of honey in wounds of stimulating the growth of tissues to repair damage. Both of these modes of action could be involved simultaneously, along with a third possibility, that of the anti-inflammatory action of honey reducing the malfunctioning of the mucosa and the loss of serum from the inflamed tissue.

The routine therapy for diarrhoea is simply re-hydrating the body and restoring electrolytes (salts) lost in the diarrhoea, by administering fluid by mouth or intravenously[64]. The World Health Organisation's recommendation for oral re-hydration is to use an electrolyte solution with glucose added[153]. The active absorption of glucose by the intestinal mucosa is a process that is coupled to the uptake of sodium[64], so the glucose aids in the absorption of electrolytes. It also increases the uptake of water[58]. In the clinical trial where honey replaced glucose in the electrolyte solution it was found that it was just as effective as glucose in re-hydrating the patients[64]. Honey has the added advantage of also containing fructose which has the ability to promote additional water uptake with less sodium uptake, avoiding the risk of too much sodium being taken up into the circulation[64]. Fructose also promotes the uptake of potassium whereas glucose causes net loss of potassium[58].

Action of honey in treating peptic ulcers and gastritis

The discovery that one of the causes of peptic ulcers and gastritis (inflammation of the stomach lining) was infection with the bacterium *Helicobacter* pylori[45] raised the suggestion that the effectiveness of honey in treating these conditions may be due to its antibacterial activity[5,14]. Testing of clinical specimens of *H.* pylori showed that they were sensitive to the antibacterial activity of honey[5,14], but possibly not sufficiently sensitive to account for the therapeutic effect of honey. The concentration of honey needed to stop the growth of the bacteria in one study[14] was 20%. In the other study[5] the bacteria were not inhibited by a 40% concentration of a honey selected to have a median level of antibacterial activity due to hydrogen peroxide, the common antibacterial component of honey. However, with a manuka honey of a median level of activity due to the unidentified antibacterial component of this type of honey, the concentration of honey needed to completely inhibit the growth of the bacteria was 5%. But a clinical trial using manuka honey with a similar level of activity has found that infection of the stomach with H. pylori was not cleared after two weeks of treatment with four-times-daily doses of a tablespoon (c. 25 g) of honey[90]. Although it was concluded from this trial that any effectiveness of honey against peptic ulcers and gastritis is not through an effect on *H. pylori,* this is not a reasonable conclusion when the trial was with only six patients treated, and was with

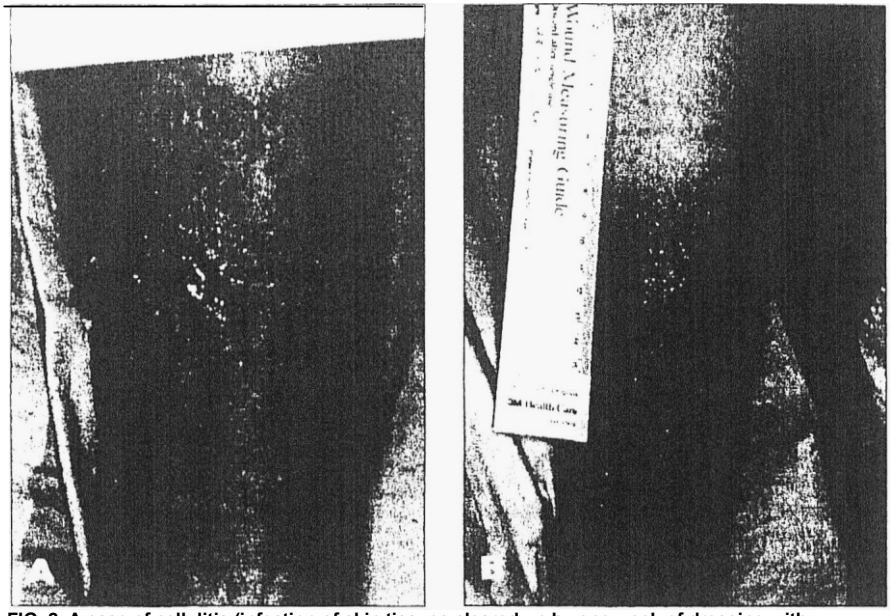

FIG. 2. A case of cellulitis (infection of skin tissues cleared up by one week of dressing with honey (A: before treatment; after).

a single, arbitrarily chosen dose rate which may have been insufficient and may not have been continued long enough to clear the infection, However, it should also be born in mind that this trial was carried out with a honey to which H. pylori is very sensitive, whereas in the many reports of successful treatment of peptic ulcers and gastritis cited in Part 1 of this review it was not manuka honey that was used.

Alternative explanations for how honey has a therapeutic effect on gastritis and peptic ulcers have come from a series of studies conducted by Ali and co-workers, who have investigated the influence of honey on various parameters known to be involved in ulceration in the stomach, There are various causes of peptic ulcers, the major ones being aspirin-type anti-inflammatory drugs, alcohol, and stress, which restricts the blood supply to the gastric mucosa (the stomach lining) and leaves it more susceptible to erosion by the stomach contents[26]. Studies of the action of honey on peptic ulcers in rats have shown that it has a dose-dependent effect protecting the stomach from ulceration being caused by alcohol[6,8,9,10,12] and indomethacin (an aspirin-type anti-inflammatory drug)[10]. At the higher dose rates used, there was around an 80% protection from the ulceration caused by alcohol[6,8], but only if the honey was given 30 minutes beforehand and not if given simultaneously. Only in one case[10], with a very high dose rate, was there any protection if the honey was given simultaneously. But honey gave 100% protection from ulceration caused by indomethacin when given simultaneously. (The difference in time frame of protection may reflect the much slower development of ulcers seen with indomethacin than with alcohol[7,8]) There was no protection from either agent if a sugar mixture simulating honey was used in place of honey[8,10], showing that the protection is due to a component of the honey other than the sugars.

Investigation by Ali *et al.* of the mechanisms of these protective effects of honey have given an insight into how honey may work in therapy of gastritis and peptic ulcers. Aspirin-type anti-inflammatory drugs, especially in the presence of acid, enter the cells and block their energy-producing metabolism, thus causing the cells to decrease their protective secretions and become permeable to ace. This leads to shedding of the surface cells and development of erosion of the sub-surface, with bleeding and inflammation[26]. Production of prostaglandins, with a protective function, is inhibited by these drugs, but prostaglandins protect only the sub-surface mucosal tissue, repair of the mucosal surface (epithelial cells) being independent of prostaglandins[26]. The action of alcohol is more complex and less well understood, but also involves inflammation[9,10].

The studies on the effects of honey on ulcers have demonstrated that an influence of honey on prostaglandin production is not involved[6,9], but that honey has a stimulatory effect on the sensory nerves in the stomach that respond to capsaicin (the irritant in chilli pepper)[6]. Stimulation of these nerves causes the release of vasodilatory peptides in the stomach which, mediated by production of nitric oxide, increase the blood supply and thus help protect the gastric mucosa from damage[6,11].

A second mechanism of action has also been identified from these studies that involves the antioxidant properties of honey. Honey has been found to protect or augment the level of non-protein sulfhydryls (substances such as glutathione) in gastric tissue subjected to factors inducing ulceration[6,8,9,13], a class of substances that are part of the body's antioxidant defence system[65], and depletion of which is an indication of oxidative damage to tissues[39]. Oxidative damage to tissues through free radical production occurs in reperfusion injury (injury resulting from the restoration of blood flow to tissues that have been deprived of it). The free radicals are formed by the action of the enzyme xanthine oxidase in the tissues, formed during the period of oxygen starvation, producing superoxide from oxygen when it becomes available again[39]. This type of injury is involved in the formation of peptic ulcers[13], and has been found to be decreased in rat stomachs by dosing with honey 30 minutes before restricting then restoring the circulation[13]. Another study showed that the permeability of the blood vessels in the gastric mucosa developing as a consequence of exposure to alcohol, a feature of inflammation, could be reduced in a dose-dependent manner by pretreatment of the stomach with honey[12]. But none of these findings of an antioxidant effect of honey in the stomach rule out the alternative or additional possibility that it is an antiinflammatory component of honey distinct from the antioxidants that is involved. As mentioned above, oxygen free radicals can initiate further inflammation, and inflammation gives rise to oxygen free radicals, thus giving a self-amplifying inflammatory response[56]. The oxidative damage resulting could be decreased by blocking either the oxygen radicals themselves, or by blocking the inflammatory response that would otherwise be giving rise to more oxygen radicals.

Ali et *al.* have also identified a third mechanism of action of honey in the therapy of peptic ulcers, that of stimulating repair of the damage to the gastric mucosa. Feeding honey to rats with stomach ulcers caused by indomethacin gave 61-70% more healing than in the controls[7]. Observation of the ulcers revealed that the honey caused a decrease in oedema (swelling of the surrounding tissue, a feature of inflammation) and formation of healthy granulation tissue.

It is of interest that these observations parallel those made with skin ulcers treated with honey (see above). Ali et *al.* have proposed[7] that the stimulation of healing of peptic ulcers is by its stimulation of blood supply[6,11], which is one of the mechanisms that is involved in the healing of skin ulcers (see above). The anti-inflammatory action reducing oedema would be involved in this as well (see above), additional to the direct stimulation through the sensory nerves in the stomach. The stimulatory effect of honey on the growth of epithelial cells (see above) could also be expected to help restore the surface cells of the gastric mucosa, which cannot be helped by prostaglandins.

The role of hydrogen peroxide

Hydrogen peroxide, the principle antibacterial component of honey, is well known as an antibacterial agent, although it has had a chequered history of use as an antiseptic. In its history it has been in then out of favour with the medical profession twice since first coming into use in the late 19th Century. It has been suggested that its ready decomposition in solutions containing traces of catalytic metals such as iron or copper may be the reason why hydrogen peroxide went out of favour as an antiseptic after initially being hailed for its antibacterial and cleansing properties when first introduced[140]. There was an upsurge of interest in its use later when stabilized preparations became available, with good germicidal activity being reported[140], but in more recent times it has again gone out of favour as awareness has developed of the inflammation and damage that are caused to tissues by substances giving rise to oxygen free radicals[65,118,119]. However, the hydrogen peroxide concentration produced in honey activated by dilution is typically around 1 mmo1/1[3], about one thousand times less than in the 3% solution that is commonly used as an antiseptic. Also, there is the potential for honey to sequester and inactivate the metal ions which catalyse the formation of oxygen radicals from hydrogen peroxide, and the antioxidant components of honey to mop up any free radicals that may be formed.

Hydrogen peroxide is an effective antimicrobial agent if present at a sufficiently high concentration[116], but at higher concen -trations causes cellular and protein damage in tissues by giving rise to oxygen radicals[36,125]. A study of hydrogen peroxide antiseptic has found that there is no bactericidal concentration of hydrogen peroxide that is not toxic to fibroblasts (the cells that repair wounds)[87]. Minimum concentrations reported to be necessary in the culture medium to inhibit bacterial growth range from 0.12 to 5.9 mmol/l[92], However, it has been reported that a given quantity of hydrogen peroxide is more effective when it is supplied by continuous generation by glucose oxidase than when it is added separately', and a study with *Escherichia coil* exposed to a constantly replenished stream of hydrogen peroxide showed that their growth was inhibited by 0.02-0.05 mmo1/1 hydrogen peroxide, a concentration that was not damaging to fibroblast cells from human skin[75]. A further consideration is that myeloperoxidase, the enzyme that generates bacteria-destroying free radicals from hydrogen peroxide in the phagocytotic vacuoles of the leucocytes[83], is inactivated by hydrogen peroxide levels in excess of 2 mmo1/1[3]. Thus, in living tissue where there will be leucocytes active, a better overall antibacterial action may be obtained with low levels of hydrogen peroxide. The action of the enzymes catalase and glutathione peroxidase in tissues will give equilibrium concentrations of hydrogen peroxide that will be lower than the 1 mmo1/1 found in honey solutions *in vitro.*

But hydrogen peroxide has roles in healing quite separate from any antibacterial action. It has been reported that at levels of 30-100 pmo1/1 it activates the NF-KB transcription factor in lymphocytes to activate the expression of genes for the immune response[121]. Research on various cell lines in culture is showing that it has a variety of effects in the role of a 'cellular messenger'. A review of the voluminous literature appearing on this topic[29] has pointed out the large amount of evidence for hydrogen peroxide being involved in many cell types in the body as a stimulus for cell multiplication. It acts at various points in the mechanisms of the cells that control the cycle of cell growth and division, most probably by oxidising the proteins involved and thus causing a change in the conformation of the protein molecule. This action has particular relevance in wound healing, where the inflammatory response that is a natural consequence of injury or infection produces hydrogen peroxide, and this serves to stimulate the growth of fibroblasts and epithelial cells to repair the damage[29]. Only where there is excessive inflammation does the hydrogen peroxide rise to levels that instead cause destruction of tissues by killing the cells[29]. Even with these high levels of hydrogen peroxide the cells can be protected by iron-chelating agents which prevent the catalysis by iron of the formation of membrane-damaging free radicals[29]. Without this protection, hydrogen peroxide is toxic to cells at concentrations above 0.1 mmo1/1, but only needs to be at levels around one thousandth of this to stimulate cell multiplication[29]. It has been proposed that low concentrations of hydrogen peroxide might be used to stimulate wound healing, rather than the expensive cell growth factors produced by biotechnology for this purpose (the bioactive wound dressings)[29]. But another proposal that hydrogen peroxide could be applied to promote the wound healing process has pointed out that this is feasible only if the concentration could be carefully controlled[34]. It has also been proposed that honey be used in place of recombinant growth factors to provide hydrogen peroxide to stimulate the healing of burns[112]. The application of creams containing hydrogen peroxide to stimulate the development of new capilliaries in wound tissue[139]. It is possibly through the production of hydrogen peroxide in the presence of components protecting the cells from oxidative damage that honey is effective in stimulating the rate of healing, and particularly in kick-starting the healing process in wounds that have remained unhealed for a long time.

Another cell growth factor involved in wound healing is the hormone insulin. Wound healing research has shown that intravenous infusion of insulin or applying it to the surface of a wound stimulates the rate of healing[19,89,109]. This is to be expected, as when insulin is present it binds to the insulin receptor protein molecules on the outside of cells and causes them to change conformation, thus triggering a chain of molecular events in the cell that stimulates the uptake of glucose and amino acids, and promotes anabolic metabolism, giving cell growth. The insulin receptor complexes are activated in the same way by low concentrations of hydrogen peroxide[41,72,84], raising the possibility that this is another mechanism by which honey may stimulate wound healing.

Change in the conformation of protein molecules brought about by oxidation by hydrogen peroxide may account for another feature of honey seen when it is used on wounds, that of enzymic debridement of the wound. Although any moist dressing promotes the removal of pus and dead tissue by allowing the action of protein-digesting enzymes in the wound tissues, this debriding action by honey is remarkable. There are two types of protein-digesting enzyme involved in wound tissues: the matrix

metalloproteases of the connective tissue[99], and the serine proteases produced by the neutrophil leucocytes[138]. The serine pro-teases are normally inactive because of the presence of an inhibitor, but hydrogen peroxide inactivates the inhibitor, so the protease becomes active[106]. The metalloproteases are normally present in an inactive conformation, but hydrogen peroxide changes the conformation of these and makes them active[107,148].

Conclusions

Although honey has in the past been a standard medicine, most medical practitioners in the present day in developed countries are not aware of that, and consider it to be an 'alternative' or 'complementary' medicine. Although there are some very good indications of its effectiveness in reports published in medical journals, there is evidence from randomized controlled clinical trials only for its use as a dressing for burns. Even where there is evidence of effectiveness there is still a reluctance to use alternative medicines where there is no rational explanation for how they work. Thus, it is unlikely that the further randomized controlled clinical trials necessary to conclusively establish the effectiveness of honey as a medicine, and discover how it compares in performance with modern pharmaceuticals will be carried out. This review of the literature has shown that there are rational explanations for the therapeutic effects of honey. But further research is needed to establish that the possible explanations deduced from other biomedical research findings are in fact what is occurring when honey is used.

In any future research, the large variation in composition of honey needs to be taken into account. There has been a tendency in the past to consider any honey to be representative of all honey, and the consequence of this is seen in the very large differences in findings reported on the sensitivity of bacteria to honey[92,93]. In Part 2 of this review mention was made of the awareness of the ancient physicians, and in present day folk medicine, of particular honeys being the best for particular medical uses, yet no account of this is taken in any of the clinical trials of honey. Considerations in the selection of honey for medical use have been discussed[95], and the point raised that until the importance of the anti-inflammatory and antioxidant components of honey have been established, only the antibacterial activity of honey for use as a medicine can be standardized. In light of the likely importance of all of these components, the need for further research to identify their involvement and their nature is needed, so that honeys can be selected to give the best results when used as a medicine.

References

1. ABBAS, T (1997) Royal treat. *Living in the Gulf* pp 50-51.
2. ABUHARFEIL, N; AL-ORAN, R; ABO-SHEHADA, M (1999) The effect of bee honey on the proliferative activity of human B- and T-lymphocytes and the activity of phagocytes. *Food and Agricultural Immunology* 11: 169-177.
3. AGNER, K (1963) Studies on myeloperoxidase activity. 1. Spectrophotometry of the MPO-H202 compound. *Acta Chemka Scandinavica* 17(Suppl. 1): S332–S338.
4. AKHTAR, M 5; KHAN, M S (1989) Glycaemic responses to three different honeys given to normal and alloxan-diabetic rabbits. *Journal of* the *Pakistan Medical Association* 39(4):107-113.
5. AL SOMA!, N; COLEY, K E; MOLAN, P C; HANCOCK, B M (1994) Susceptibility of Helkobocter pylori to the antibacterial activity of manuka honey. *Journal of the Royal Society* of *Medicine* 87(1): 9-12.
6. AL-SWAYER. 0 A; ALI, A T M (1998) Effect of ablation of capsaicin-sensitive neurons on gastric protection by honey and sucralfate. Hepato-Gastmencerology 45(19): 297-302,
7. ALI, A T M (1995) Natural honey accelerates healing of indomethacin-induced antral ulcers in rats. *Saudi Medical Journal* 16(2): 161-166.

8. ALI, A TM M (1991) Prevention of ethanol-induced gastric lesions in rats by natural honey, and its possible mechanism of action. *Scandinavian Journal of Gastroenterology* 26. 281-288.
9. ALI, A TM M (1995) Natural honey exerts its protective effects against ethanol- induced gastric lesions in rats by preventing depletion of glandular nonprotem sulfhydryls. Tropical *Gastroenterology* 16(1): 18-26.
10. ALI, A T M M; AL-HUMAYYD, M S; MADAN. B R (1990) Natural honey prevents indomethacin- and ethanol-induced gastric lesions *in* rats. *Saudi Medical Journal* 11(4): 275-279.
11. ALI, ATM M; AL-SWAYEH, 0 A (1996) The role of nitric oxide in gastric protection by honey. *Saudi Medical Journal* 17: 301-306.
12. ALI, ATM M; AL-SWAYEH, 0 A (1997) Natural honey prevents ethanol-induced increased vascular permeability changes in the rat stomach. *Journal of Ethnopharmacology* 55(3): 231-238.
13. ALI, A T M M; AL-SWAYEH, 0 A; AL. HUMAYYD, M 5; MUSTAFA, A A; AL-RASHED, R S; AL-TUWAIJIRI, A S (1997) Natural honey prevents ischaernia.reprfuston-induced gastric mucosal lesions and increased vascular permeability in rats. European Journal *of Gastroenterology and* Hepatology 9(11): 1101-1107.
14. ALI, A T M M; CHOWDHURY, M N H; AL HUMAYYD, M S (1991) Inhibitory effect of natural honey on Helicobocter pylon. *Tropical Gastroenterology* 12(3): 139-143.
15. ALLEN, K L; MOLAN, P C (1997) The sensitivity of mastitis-causing bacteria to the antibacterial activity of honey. *New Zealand* Journal of *Agricultural Research* 40: 537-540.
16. ARISTOTLE ((350 BC) 1910) *Historic Animohum,* Oxford University; Oxford. UK.
17. ARMON, P J (1980) The use of honey in the treatment of infected wounds. *Tropical* Doctor 10: 91.
18. BAUER, L; KOHLICH, A; HIRSCHWEHR. R; SIEMANN, LI; EBNER, H; SCHEINER, 0; KRAFT, D; EBNER, C (1996) Food allergy to honey: pollen or bee products? Characterisation of allergenic proteins in honey by means of immunoblotting. *Journal of Allergy and Clinical* Immunology 97(1): 65-73
19. BELFIELD, W 0; GOLINSKY. S; COMPTON, M D (1970) The use of insulin in open wound healing. Veterinary *Medicine:* Small *Animal Clinician* 65(5): 455-460.
20. BERGMAN. A; YANAI,); WEISS. J: BELL. D; DAVID, M P (1983) Acceleration of wound healing by topical application of honey An animal model. *American Journal of Surgery 145'* 374-376.
21. BLOMFIELD, R (1973) Honey for decubitus ulcers *Journal of* the *American Medical Association* 224(6): 905.
22. BLOOMFIELD. E (1976) Old remedies. *Journal of the Royal College of General Practitioners* 26.576.
23. BOSE, B (1982) Honey or sugar in treatment of infected wounds? *Lancet* i(April 24): 963
24. BRADY, N F; MOLAN, P C; HARFOOT, C G (1997) The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey. *Pharmaceutical Sciences* 2: 1-3.
25. BRANIKI, F (1981) Surgery in Western Kenya. *Annals of the Royal College of Surgeons of England* 63: 348-352.
26. BROOKS, F P (1985) The pathophysiology of peptic ulcer disease. Digestive Diseases *and* Sciences 30(1 1): 15S-29S
27. BUCKNALL, T E (1984) Factors affecting healing *In* T E Bucknall; H Ellis (eds) *Wound healing* for *surgeon*. BailliEre Tindall; London, UK; pp 42-74
28. BULMAN, M *W* (1955) Honey as a surgical dressing. *Middlesex* Hospital *Journal* 55. 188-189.
29. BURDON, R H (1995) Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radical* Biology *and Medicine* 18(4): 77S-794.
30 BURLANDO, F (1978) Sull'azione cerapeutica del miele nelle ustioni. *Minerva Dermatologico* 113. 699-706
31. CAVANAGH, D; BEAZLEY, J; OSTAPOWICZ. F (1970) Radical operation for carcinoma of the vulva. A new approach to wound healing *Journal of* Obstetrics *and Gynaecology* of the *British* Commonwealth 77(11): 1037-1040.
32. CHANT, A (1999) The biornechanics of leg ulceration. *Annals* of *the Royal College of Surgeons of England* 81.80-85.
33. CHIRIFE, J; HERSZAGE, L; JOSEPH, A; KOHN. E S (1983) *In vitro* study of bacterial growth inhibition in concentrated sugar solutions. microbiological basis for the use of sugar in treating infected wounds. *Antimicrobial Agents and Chemotherapy* 23(5): 766-773.
34. CHUNG. L Y; SCHMIDT, R J; ANDREWS. A M: TURNER, T D (1993) A study of hydrogen peroxide generation by, and antioxidant activity of, Granuflex''"' (DuoDERM-') Hydrocolloid Granules and some other hydrogel/hydrocolloid wound management materials. *British journal* of Dermatology 129(2): 14S-53.

35. CHURCH, J (1954) Honey as a source of the anti-stiffness factor. Federation Proceedings of *the American Physiology* Society 13(1): 26.

36, COCHRANE, C G (1991) Cellular injury by oxidants. *American Journal of Medicine* 91(Suppl. 3c): 23S-30S.

37. COOPER, R A; MOLAR P C (1999) The use of honey as an antiseptic in managing Pseudomonas infection. *Journal of Wound Care 8(4):* 161-164.

38. COOPER, R A; MOLAR P C; HARDING, K G (1999) Antibacterial activity of honey against strains of Staphylococcus *oureus* from infected wounds.Joumal *of the Royal* Society of Medicine 92: 283-285.

39. CROSS, C E; HALLIWELL, B; BORISH, E T; PRYOR. W A; AMES, B N; SAUL, R L; MCCORD, J M; HARMAN, D (1987) Oxygen radicals and human disease. *Annals of Internal Medicine* 107: 526-545.

CURDA, L; PLOCKOV•, M (1995) Impedance measurement of growth of lactic acid bacteria in dairy cultures with honey addition, *International Dairy Journal* 5: 727-733.

41. CZECH, M LAWRENCE JR, J C; LYNN, W S (1974) Evidence for the involvement of sulphydryl oxidation in the regulation of fat cell hexose transport by insulin. *Proceedings* of the *National Academy* of Sciences of the United States *of* America 71(10): 4173-4177.

42. DAILEY, L A; IMMING, P (1999) 12-Lipoxygenase: classification, possible therapeutic benefits from inhibition, and inhibitors. *Current Medical Chemistry 6(5):* 389-398.

43. DAVIS, C; ARNOLD, K (1974) Role of meningococcal endotoxin in meningococcal purpura. *Journal* of *Experimental Medicine* 140: 159-171.

44. DEFORGE, L E; FANTONE, J C; KENNEY, J 5; REMICK, D G (1992) Oxygen radical scavengers selectively inhibit interleukin 8 production in human whole blood. Journal of *Clinical Investigation* 90: 2123-2129.

45. DOOLEY, C P; COHEN, H (1989) The clinical significance of Campylobacter pylori. *Annals* of *Internal Medicine* 108: 70-79.

46. DUMRONGLERT, E (1983) A follow-up study of chronic wound healing dressing with pure natural honey. Journal of the *National Research Council of Thailand* 15(2): 39-66.

47. DUNFORD, C: COOPER. R A; MOLAR P C (2000) Using honey as a dressing for infected skin lesions. *Nursing* Times 96(NTPLUS 14): 7-9.

48. DUNFORD, C; COOPER, R A; WHITE, R J; MOLAR, P C (2000) The use of honey in wound management. *Nursing Standard* 15(11): 63-68.

49. EFEM, S E E (1988) Clinical observations on the wound healing properties of honey. *British Journal* of Surgery 75: 679-681.

50. EFEM, S E E (1993) Recent advances in the management of Fournier's gangrene: preliminary observations. *Surgery* 113(2): 200-204.

51. EFEM, S E E; Udoh, K T; Iwara. C I (1992) The antimicrobial spectrum of honey and its clinical significance. *Infection* 20(4): 227-229.

52. EL-BANBY, M; KANDIL, A; ABOU-SEHLY, G; EL-SHERIF, M E; ABDEL-WAHED, K. Healing effect of floral honey and honey from sugar-fed bees on surgical wounds (animal model). *In* IBRA (eds) *4th International Conference on Apicuhure in* Tropical *Climates, 1989, Cairo.* International Bee Research Association; Cardiff, UK.

53. EL-SUKHON, S N; ABU-HARFEIL, N; SALLAL, A K (1994) Effect of honey on bacterial growth and spore germination. *Journal of* Food Protection 57(10): 918-920.

54. EMARAH, M H (1982) A clinical study of the topical use of bee honey in the treatment of some occular diseases. *Bulletin of Islamic Medicine 2(5):* 422-425.

55. FAROUK, A; HASSAN, T; KASHIF, H; KHALID, S A; MUTAWAU, I; WADI, M (1988) Studies on Sudanese bee honey: laboratory and clinical evaluation. *International Journal of Crude Drug Research* 26(3): 161-168.

56. FLOHE, L; BECKMANN. R; GIERTZ, H; LOSCHEN, G (1985) Oxygen-centred free radicals as mediators of inflammation. *In* H Sies (ed) *Oxidative Stress.* Academic Press; London, UK; pp 403-435.

57. FLORIS, I; PROTA, R (1989) Sul miele amaro di Sardegna. Apkokore Modemo 80(2): 55-67.

58. FORDTRAR J S (1975) Stimulation of active and passive sodium absorption by sugars in the human jejunum. *Journal of Clinical Investigation* 55: 728-737.

59. FOTIDAR, M R; FOTIDAR, S N (1945) 'Lotus' honey. *Indian Bee Journal 7:* 102.

60. FRANKEL, S; ROBINSON, G E; BERENBAUM, M K (1998) Antioxidant capacity and correlated characteristics of 14 unifloral honeys. Journal of *Apicultural Research* 37(1): 27-31.

61. GRIMBLE, G F (1994) Nutritional antioxidants and the modulation of inflammation: theory and practice. New *Horizons 2(2):* 175-185.

62. GUNTHER, R T (1934 (Reprinted 1959)) *The Greek herbal of Dioscorides.* Hafner; New York; 701 pp.

63. GUPTA. S K; SINGH, H; VARSHNEY, A C; PRAKASH, P (1992) Therapeutic efficacy of honey in infected wounds in buffaloes. *Indian Journal* of Animal *Sciences* 62(6): 521-523.
64. HAFFEJEE, I E; MOOSA, A (1985) Honey in the treatment of infantile gastroenteritis. *British Medical Journal* 290: 1866-1867.
65. HALLIWELL, B; CROSS, C E (1994) Oxygen-derived species: Their relation to human disease and environmental stress. *Environmental Health Perspectives* 102 Suppl 10: 5-12.
66. HARRIS, S (1994) Honey for the treatment of superficial wounds: a *case* report and review. Primary *Intention 2(4):* 18-23,
67. HASPOLAT, K; BOYOKBAS, 5; «ENGEL, H (1990) Balin in vitro antibakteriyel ve antifungal etkisi. *Turk Hijiyen ve Deneysel Biyoloji Dergisisi* 47(2); 211-216.
68. HAURY, B; RODEHEAVER, G; VENSKO, *J;* EDGERTON, M T; EDLICH, R F (1978) Debridemenc an essential component of traumatic wound care. *American Journal of Surgery* 135: 238-242.
69. HAYDAK, M H (1955) The nutritional value of honey. *American Bee Journal* 95: 185-191.
70. HEJASE, M J; E., S J; BIHRLE, R; COOGAN, C L (1996) Genital Fournier's gangrene: experience with 38 patients. Urology 47(5): 734-739.
71. HELBUNG, A; PETER. C; BERCHTOLD, E; BOGDANOV, S; MULLER, U (1992) Allergy to honey: relation to pollen and honey bee allergy. *Allergy* 47(1): 41-49.
72. HELM, B A; GUNN, J M (1986) The effect of insulinomimetic agents on protein degradation in H35 hepatoma cells. *Molecular and Cellular Biochemistry* 71(2): 159-166.
73. HUNT, T K; TWOMEY, P; ZEDERFELDT, B; DUNPHY, J E (1967) Respiratory gas tensions and pH in healing wounds. *American Journal of Surgery* 114: 302-307.
74. HUTTON, D J (1966) Treatment of pressure sores. *Nursing Times* 62(46): 1533-1534.
75. HYSLOP, P A; HINSHAW, D B; SCRAUFSTATTER, I U; COCHRANE. C G; KUNZ, S; VOSBECK, K (1995) Hydrogen peroxide as a potent bacteriostatic antibiotic: implications for host defense. Free Radical *Biology and Medicine* 19(1): 31-7.
76. JONES, K P; BLAIR, S; TONKS, A; PRICE, A: COOPER, R (2000) Honey and the stimulation of inflammatory cytokine release from a monocytic cell line. *First World Wound Healing Congress;* Melbourne, Australia.
77. KANDIL, A; EL-BANBY, M; ABDEL-WAHED, K; ABOU-SEHLY, G; EZZAT N (1987) Healing effect of true floral and false nonfloral honey on medical wounds, *Journal* of *Drug Research (Cairo)* 17(1-2): 71-75.
78. KATSILAMBROS, N I; PHILIPPIDES, P; TOULIATOU, A; GEORGAKOPOULOS, K; KOFOTZOULI, FRANGAKI, D; SISKOUDIS, P; MARANGOS, M; SFIKAKIS, P (1988) Metabolic effects of honey (alone or combined with other foods) in Type II diabetics. *Aaa Diabetologica Latina* 25: 197-203.
79. KAUFMAN, T; EICHENLAUB, E H; ANGEL M F; LEVIN, M; FUTRELL, J W (1985) Topical acidification promotes healing of experimental deep partial thickness skin burns: a randomised double-blind preliminary study. *Burns* 12: 84-90.
80. KAUFMAN, T; LEVIN, M; HURWITZ, D J (1984) The effect of topical hyperalimentation on wound healing rate and granulation tissue formation of experimental deep second degree burns in guinea-pigs. *Burns* 10(4): 252-256,
81. KEAST-BUTLER, J (1980) Honey for necrotic malignant breast ulcers. *Lancet* ii(October 11): 809.
82. KIISTALA, R; HANNUKSELA, M; MAKINEN-KILJUNEN, S; NIINIMAKI, A; HAAHTELA, T (1995) Honey allergy is rare In patients sensitive to pollens. *Allergy* 50: 844-847.
83. KLEBANOFF, S J (1980) Myeloperoxidase-mediated cytotoxic systems. In A J Sbarra; R • R Strauss (eds) The *reticuloendothelial system. A comprehensive treatise. Volume 2. Biochemistry and Metabolism.* Plenum Press; New York: pp 270-308.
84. KOSHIO, 0; AKANUMA, Y; KASUGA, M (1988) Hydrogen peroxide stimulates tyrosine phosphorylation of the insulin recepter and its tyrosine kinase activity In intact cells, *Biochemical Journal* 250: 95-101.
85. KUMAR, A; SHARMA, V K; SINGH, H P: PRAKASH, P; SINGH, S P (1993) Efficacy of some indigenous drugs in tissue repair in buffaloes. *Indian VeterinaryJaimal* 70(1): 41444,
86, LEVEEN, H H; FALK, G; BOREK, B; DIAZ, C; LYNFIELD, Y; WYNKOOP B J; MABUNDA, G A; RUBRICUS, J L CHRISTOUDIAS, G C (1973) Chemical acidification of wounds. An adjuvant to healing and the unfavourable action of alkalinity and ammonia. *Annals of Surgery* 178(6): 745-753.
87. LINEAWEAVER, W; MCMORRIS, S; SOUCY, 0; HOWARD, R (1985) Cellular and bacterial toxicities of topical antimicrobials. *Plastic and Reconstructive Surgery* 75(3): 394-396.

88. LINNETT, P. (1996) Honey for equine diarrhoea. Control *and* Therapy: 906.
89. LOPEZ, J E; MENA, B (1968) Local insulin for diabetic gangrene. Lancet i: 1199.
90. MCGOVERN, D P B; ABBAS, S Z; VIVIAN. G; DALTON, H R (1999) Manuka honey against Helicobacter *pylori. Journal of the Royal* Society of *Medicine* 92: 439.
91. MCINERNEY, R J F (1990) Honey - a remedy rediscovered. *Journal of the Royal* Society of *Medicine* 83: 127.
92. MOLAN, P C (1992) The antibacterial activity of honey. 1. The nature of the antibacterial activity. Bee *World* 73(1): 5-28.
93. MOLAN, P C (1992) The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. Bee *World* 73(2): 59-76.
94. MOLAN, P C (1998) A brief review of honey as a clinical dressing. *Primary Intention 6(4):* 148-158.
95. MOLAN, P C (1999) Selection of honey for use as a wound dressing. *Primary Intention* (in press).
96. MOLAN, P C (1999) Why honey is effective as a medicine. I. Its use in modern medicine. Bee *World* 80(2): 80-92.
97. MOLAN, P C; Allen, K L (1996) The effect of gamma-irradiation on the antibacterial activity of honey.foumal of Pharmacy *and Pharmacology* 48: 1206-1209.
98. MOSSEL, D A A (1980) Honey for necrotic breast ulcers, Lancet ii(November 15): 1091.
99. MURPHY, G; REYNOLDS, J J; BRETZ, U; BAGGIOLINI, M (1982) Partial purification of coilagenase and gelatinase from human polymorphonuclear leukocytes. Biochemical *Journal* 203: 209-221.
100. MURRELL, G A C; FRANCIS. M J 0; BROMLEY, L (1990) Modulation of fibroblast proliferation by oxygen free radicals. *Biochemical Journal* 265: 659-665.
101. NDAYISABA, G; BAZIRA, L.; HABONIMANA, E; MUTEGANYA, D (1993) Clinical and bacteriological results in wounds treated with honey. *Journal of Orthopaedic Surgery 7(2):* 202-204.
102. NIINIKOSKI, J; KIVISAARI, J; VILJANTO, J (1977) Local hyperalimentation of experimental granulation tissue. *Acta* Chiropida *Scandinavica* 143: 201-206.
103. NYCHAS, G J; DILLON, V M; BOARD, R G (1988) Glucose, the key substrate in the microbiological changes in meat and certain meat products. Biotechnology *and Applied Biochemistry* 10: 203-231.
104. OBI, C L; UGOJI, E 0; EDUN, S A; LAWAL, S F; ANYIWO, C E (1994) The antibacterial effect of honey on diarrhoea causing bacterial agents isolated in Lagos, Nigeria. *African Journal of Medical* Sciences 23: 257-260.
105. ORYAN, A; ZAKER, S R (1998) Effects of topical application of honey on cutaneous wound healing in rabbits. *Journal* of *Veterinary Medicine, Series A* 45(3): 181-188.
106. OSSANNA, P J; TEST, S T; MATHESON, N R; REGIANI, 5; WEISS, S J (1986) Oxidative regulation of neutrophil elastase-alpha-1-proteinase inhibitor Interactions. *Journal of Clinical Investigation 77:* 1939-1951.
107. PEPPIN, G *J;* WEISS, S J (1986) Activation of the endogenous metalloproteinase, gelatinase, by triggered human neutrophiis. Proceedings of the *National Academy* of Sciences of the *United States of America* 83: 4322-4326,
108. PHUAPRADIT. W; SAROPALA. N (1992) Topical application of honey in treatment of abdominal wound disruption. *Australian and New Zealand Journal of Obstetrics and Gynaecology* 32(4): 381-384.
109. PIERRE, E J; BARROW, R E; HAWKINS, H K; NGUYEN, T T; SAKURAI, Y; DESAI, M; WOLFE, R R; HERNDON, D N (1998) Effects of insulin on wound healing. *Journal of Trauma, Injury, Infection and Critical Care* 44(2): 342-345.
110. POSTMES, T; BOGAARD, A E VAN DEN; HAZEN, M (1993) Honey for wounds, ulcers, and skin graft preservation. Lancet 341(8847): 756-757,
111. POSTMES, T; BOGAARD, A E VAN DEN; HAZEN, M (1995) The sterilization of honey with cobalt 60 gamma radiation: a study of honey spiked with Clostridium *botulinum* and *Bacillus subtilis. Experentia (Basel)* 51: 986-989,
112. POSTMES, T; VANDEPUTTE, J (1999) Recombinant growth factors or honey! *Burns* 25(7): 676-678.
113. POSTMES, T J; BOSCH, M M C; DUTRIEUX, R; BAARE, J VAN; HOEKSTRA, M J (1997) Speeding up the healing of burns with honey. An experimental study with histological assessment of wound biopsies. In A Mizrahi; Y Lensky (eds) Bee products: properties, *applications and apitherapy.* Plenum Press; New York; pp 27-37.
114. PRUITT, K M; REITER, B (1985) Biochemistry of peroxidase system: antimicrobial effects. *In* K M Pruitt: J 0 Tenovuo (eds) *The lactoperoxidase system: chemistry and biological significance.* Marcel Dekker; New York; pp 144-178.

115. ROOS, D (1991) The respiratory burst of phagocytic leucocytes. *Drug Investigation* 3(suppl. 2): 48-53.

116. ROTH, L A; KWAN, S; SPORNS, P (1986) Use of a disc-assay system to detect oxytetracycline residues in honey. *Journal* of Food Protection 49(6): 436-441.

117. RYAN, G B; MAJNO, G (1977) Inflammation. Upjohn; Kalamazoo, Michigan, USA; 80 pp.

118. SASSY, J M; GUIGNARD, B; PATS, B; GUIAVARCH, M; ROUVIER, B (1995) Pulmonary edema after hydrogen peroxide irrigation of a war wound. *Intensive Care Medicine* 21(3): 287-288.

119. SALAHUDEEN, A K; CLARK, E C; NATH, K A (1991) Hydrogen peroxide-induced renal injury. A protective role for pyruvate *in vitro* and *in vivo. Journal* of *Clinical Investigation* 88(6): 1886-1893.

120. SAMANTA, A; BURDEN, A C; JONES, G R (1985) Plasma glucose responses to glucose, sucrose, and honey in patients with diabetes mellitus: an analysis of glycaemic and peak incremental indices. Diabetic *Medicine 2(5):* 371-373.

121. SCHRECK, R; RIEBER, P; BAEUERLE, P A (1991) Reactive oxygen Intermediates as apparently widely used messengers in the activation of the NF-kB transcription factor and HIV-1, EM80 *Journal* 10(8): 2247-2258.

122. SHEIKH, D; SHAMS-UZ-ZAMAN; NAQVI, S B; SHEIKH, M R; ALI, G (1995) Studies on the antimicrobial activity of honey. Pakistan *Journal* of Pharmaceutical Sciences 8(1): 51-62.

123. SILVER, I A (1980) The physiology of wound healing. *In* T K Hunt (ed) *Wound healing and wound infection: theory and surgical practice.* Appleton-Century-Crofts; New York; pp 11-28.

124. SILVETTI, A N (1981) An effective method of treating long-enduring wounds and ulcers by topical applications of solutions of nutrients. *Journal of Dermatolology, Surgery and Oncology 7(6):* 501-508,

125. SIMON, R H; SCOGGIN, C H; PATTERSON, D (1981) Hydrogen peroxide causes the fatal injury to human fibroblasts exposed to oxygen radicals. *Journal of Biological Chemistry* 256(14): 7181-7186.

126. SINCLAIR, R D; RYAN, T J (1994) Proteolytic enzymes in wound healing the role of enzymatic debridement *Australasian Journal of* Dermatology 35: 35-41.

127. SOMERFIELD, S D (1991) Honey and healing. *Journal* of the *Royal Society of Medicine* 84(3): 179.

128. SUBRAHMANYAM, M (1991) Topical application of honey in treatment of burns. *British Journal* of *Surgery* 78(4): 497-498.

129. SUBRAHMANYAM, M (1993) Honey impregnated gauze versus polyurethane film (OpSite(r)) in the treatment of burns - a prospective randomised study. British *Journal* of *Plastic Surgery* 46(4): 322-323.

130. SUBRAHMANYAM, M (1994) Honey-impregnated gauze versus amniotic membrane in the treatment of burns. *Burns* 20(4): 331-333.

131. SUBRAHMANYAM, M (1996) Honey dressing versus boiled potato peel in the treatment of burns: a prospective randomized study. Burns 22(6): 491-493.

132. SUBRAHMANYAM, M (1998) A prospective randomised clinical and histological study of superficial bum wound healing with honey and silver sulfadiazine. Bums 24(2): 157-161.

133. SUGUNA, L; CHANDRAKASAN, G; RAMAMOORTHY, U; THOMAS JOSEPH, K (1993) Influence of honey on biochemical and biophysical parameters of wounds in rats. *Journal of Clinical Biochemistry and Nutrition* 14: 91-99.

134. SUGUNA, L; CHANDRAKASAN, G; THOMAS JOSEPH, K (1992) Influence of honey on collagen metabolism during wound healing in rats. *Journal of Clinical Biochemistry and Nutrition* 13: 7-12,

135. SWAIM, S F (1980) *Surgery* of *traumatized skin: management and reconstruction in the dog and cat.* W B Saunders Co.; Philadelphia, USA; 120-122 pp.

136. TANAKA,1-1; HANUMADASS, M; MATSUDA, H; SHIMAZAKI, 5; WALTER, R J; MATSUDA, T (1995) Hemodynamic effects of delayed initiation of antioxidant therapy (beginning two hours after burn) in extensive third-degree burns. *Journal of Bum* Care *and Rehabilitation* 16(6): 610-615.

137. TATNALL, F M; LEIGH, I M; GIBSON, J R (1991) Assay of antiseptic agents in cell culture: conditions affecting cytotoxicity. *Journal* of Hospital *Infection* 17(4); 287-296.

138. TONNESEN, M G; WORTHEN, G 5; JOHNSTON, R B JR. (1988) Neutrophil emigration, activation and tissue damage. *In* R A F Clark; P M Henson (eds) *The molecular and cellular biology* of wound repair. Plenum Press; New York, London; pp 149-183.

139. TUR, E; BOLTON, L; CONSTANTINE, B E (1995) Topical hydrogen peroxide treatment of ischemic ulcers in the guinea pig. Blood recruitment in multiple skin *sites. Journal* of *the American Academy of Dermatology* 33(2 Pt 1): 217-221.