

THE ANTIBACTERIAL ACTIVITY OF HONEY

2. Variation in the potency of the antibacterial activity

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Introduction

Honey is gaining acceptance by the medical profession for use as an antibacterial agent for the treatment of ulcers and bed sores, and other surface infections resulting from burns and wounds^{4,135}. In many cases it is being used with success on infections not responding to standard antibiotic and antiseptic therapy. Its effectiveness in rapidly clearing up infection and promoting healing is not surprising in light of the large number of research findings on its antibacterial activity, covered in Part 1 of this review (*Bee World* 73(1): 5-28, 1992).

None of the reports in the medical literature, however, mention any selection of the honey used for the treatment of infections. Although it is recognized that honey has antibacterial activity, it is not generally realized that there is a very large variation in the antibacterial potency of different honeys, and that the antibacterial properties can be easily lost by inappropriate handling and storage of honey. Part 2 of this review covers the research that has been done on these aspects: giving regard to these findings should result in a more rational usage of honey in medicine and allow its full potential as an antibacterial agent be achieved.

Variation in antibacterial activity

A common feature of all of the reports in the medical literature on the use of honey as an antibacterial agent is that no consideration is given to the selection of type of honey for therapeutic use. Aristotle, c 350 BC⁶, and Dioscorides, c AD 50⁴⁶, recommended that honey collected in specific regions and seasons (and therefore presumably from different floral sources) be used for the treatment of different ailments. Such considerations have continued into present-day folk medicine: the strawberry-tree (*Arbutus unedo*) honey of Sardinia is valued for its therapeutic properties³⁸; in India lotus (*Nelumbium scaciosum*) honey is said to be a panacea for eye diseases³⁹. In modern clinical practice, however, these views have gone unnoticed, as have the laboratory findings of large differences in the antibacterial potency of honey from different floral sources.

Degree of variance observed

In almost all studies in which more than one type of honey has been used, differences in the antibacterial activity of the honeys have been observed. The degree of difference observed has in some cases been very large, and in many others where it has been smaller this possibly is the result of a more limited range of testing rather than of less variance in the activity of the honeys. In many studies the antibacterial activity of different honeys has been compared by way of the inhibine

number determined by the method devised by Dold and Witzhausen for such comparisons²⁹.

Dold and Witzhausen coined the term 'inhibine number' in 1955²⁹ to describe the degree of dilution to which a honey will retain its antibacterial activity. This is a term that has been widely used since as a measure of the antibacterial activity of honey. The inhibine number involves a scale of 1 to 5 representing sequential dilutions of honey in 5% steps, from 25% to 5%. There have since been various minor modifications to this method so that the actual concentration corresponding to the inhibine number reported may vary. One modification has been to estimate fractional inhibine numbers by visual assessment of partial inhibition on the agar plate with the concentration of honey that just allows growth^{1,31,108}. Another modification⁹⁶ has been the use of double-strength nutrient in the dilution mixture to keep the concentration of nutrient constant throughout the series: in the original method of Dold and Witzhausen this varied considerably. The effect of differences between methods on the comparability of the inhibine numbers from different studies has been discussed by White et al¹²⁷.

In most of the studies measuring the inhibine number of honeys, activity has been found to range over the five-fold difference in concentration in the dilution series^{1,52,61,94,96,121,122,127,126,129,130,132}. In three other studies^{21,73,108} activity was found to range over a four-fold difference in concentration in the dilution series. With some honeys not active at the highest concentration tested in some of the studies, and others still active at the greatest dilutions, it is possible that if greater and lesser degrees of dilution had been included in the testing then a wider range of activities would have been detected. One study using a wider range of dilutions (honey from 50-0.25%) found the minimum inhibitory concentrations of the honeys tested to range from 25% to 0.25%³. Another, testing from 50% to 0.4% found the minimum inhibitory concentrations to range from greater than 50% (i.e. not active at 50%) to 1.5%³⁵. Other studies with wide ranges tested also found some honeys without activity at the highest concentration tested, and other honey with activity at the lowest concentration tested: the ranges were from 20-0.6%¹⁴ and 50-1.5%^{20,57}.

When the data are examined, activities are seen to be fairly well spread over these ranges. Duisberg and Warnecke³¹ plotted the distribution of the activity of 131 samples of honey tested, and found that it deviated from a normal Gaussian distribution because of the large number of samples with low activity. (In 7% of the samples the activity was below the level of detection.) They attributed this to destruction of activity by exposure to heat and light, and estimated that 50% of the samples had lost more than half of their original activity, and 22% had lost more than three-quarters. Another study of 345 samples of honeys⁴ also found a large number with low activity (36% of the samples had activity near or below the level of detection), the rest having almost a Gaussian distribution over a twenty-fold range of activity.

Association with floral source

Although some have concluded that honey from certain plants has better antibacterial activity than that from others, there is not enough evidence for such definite conclusions to be justified. Some of these conclusions are based on data from very

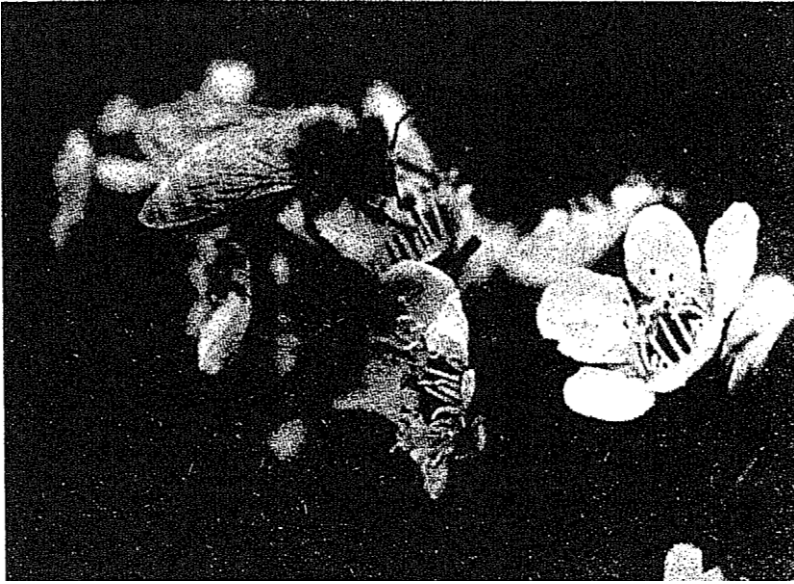


FIG. 4. Honey bee foraging on manuka flowers in New Zealand.

small numbers of samples. Other studies, though, have shown that there can be a large variation in the activity of different samples from the same plant source. Because of this, and because of the likelihood of misidentification of the source, the impossibility of getting a truly unifloral honey, and of variation associated with instability of the activity (discussed later), small numbers of samples cannot be taken as being representative of a particular source of honey. Even large-scale studies produce data of limited usefulness in this respect: because there are so many different plant species from which honey is produced, not many can be looked at from each. However, honeys from some sources have been studied in large enough numbers or have been included in enough different studies for some trends to be noted.

There have been several studies in which dark honey from the conifer forests of the mountainous regions of central Europe have been found to have particularly high activity^{14, 16, 17, 24, 25, 35, 52, 61, 81, 94, 103}. This honey is not from a nectar source, but from honeydew, produced by aphids sucking the sap from the leaves of the trees. Honey from sweet chestnut (*Castanea sativa*), a nectar source, has also been reported to have high activity^{14, 35} but it is dark in colour and thus is considered to be partly derived from honeydew¹⁴. Another dark coloured honey, from manuka (*Leptospermum scoparium*) in New Zealand (fig. 4), has also been found to have a high level of activity^{14, 61, 77, 94}. Roth et al.⁹⁰ commented on the association of high activity with dark coloured honeys in their study of Canadian honeys. Heather (*Erica* spp.) honey, which has a fairly dark colour, has been found to have a high level of antibacterial activity in one study⁴, but a fairly low^{14, 94} or low^{35, 73} level of activity in others. Rape (*Brassica napus*) honey has also been found to have a



FIG. 5. Sampling broth cultures to measure the rate of bacterial growth with different concentrations of honey added.

high level of activity in one study⁴, but a fairly low⁹⁴ or low^{35,61,77} level of activity in others. In several studies linden (*Tilia cordata*) honey has been found to have a fairly high level of activity^{14,61,77,94}, but a fairly low level of activity in others^{35,52}. Clover (*Trifolium* spp.) honey has been consistently found to have low activity^{4,14}, and cotton (*Gossypium hirsutum*) honey high activity^{103,106,128}.

Reasons for variance

The water activity of honey varies relatively little, and is not of much importance in the antibacterial effect of the dilute solutions of honey used to study the antibacterial activity of honey. Although the acidity of honey varies considerably, this too is likely to be of little consequence when the honey is in dilute solution in nutrient broth for testing its effect on bacterial cultures, as the broth buffers the acidity (fig. 5). The major variations seen in overall antibacterial activity are due to variation in the level of hydrogen peroxide achieved, and in some cases to the level of non-peroxide factors. The latter was found to be responsible for much of the activity in honeys with high levels of antibacterial activity in a study of 64 samples⁷². The content of non-peroxide factors is obviously related to the floral source, and sometimes it can account for the major part of the antibacterial activity in a honey, as is found with manuka honey⁴. The level of hydrogen peroxide achieved can also be related to the floral source, as components from some floral sources can affect both the production and the destruction of hydrogen peroxide (discussed below). There is a dynamic equilibrium: the level of hydrogen peroxide depends upon the balance between the rate of its production and the rate of its destruction¹²⁸.

Hydrogen peroxide obviously must be degraded, or else full-strength honey would contain substantial amounts of it, and any dilution of honey would eventually achieve inhibitory levels.

From the first work demonstrating that hydrogen peroxide is responsible for antibacterial activity in honey, it was realized that hydrogen peroxide is destroyed by components of honey. When testing *Staphylococcus aureus* for its susceptibility to added hydrogen peroxide, it was found that higher levels had to be added to achieve an inhibitory effect if honey was present'. Hydrogen peroxide was found to rapidly disappear when added to dilute honey, and, except in samples accumulating very high levels, the level of hydrogen peroxide accumulated from enzymatic action was seen to decline with time¹²⁸.

Of the factors possibly involved in the destruction of hydrogen peroxide, an obvious component to consider was catalase. This enzyme had long been thought to be present in honey⁹⁷, and was unequivocally shown to be present by Scheparte in 1966. Catalase comes from the pollen and nectar of certain plants; more coming from the nectar³³. honeys from some floral sources have been found to have very high levels of catalase, and these honeys accumulate low levels of hydrogen peroxide: the ones accumulating high levels of hydrogen peroxide had low levels of catalase^{33,35}. There was some deviation from the inverse correlation seen in these studies, but this could well have been the result of non-peroxide antibacterial factors giving higher levels of activity, or prior denaturation of glucose oxidase giving lower levels. The latter would probably have been the explanation for the group of honeys with low antibacterial activity and low catalase activity found in another study of 28 samples¹⁰⁰. Excluding this group, in this study a highly significant inverse correlation was found between catalase activity and accumulation of hydrogen peroxide.

Not all the variation, however, in the destruction of hydrogen peroxide associated with floral sources is due to the plants contributing catalase to the honeys. It has been found that the disappearance of hydrogen peroxide added to honey occurs even if honey is boiled beforehand to inactivate the catalase, indicating that a chemical degradation is involved as well as the enzymatic destruction¹²⁷. This could well be the metal-catalysed reaction with ascorbic acid discussed earlier

The floral source can influence the production as well as the destruction of hydrogen peroxide, thus affecting the balance between these and lowering the level of accumulation. Very large differences have been found between honeys from different floral sources in the thermal stability of their glucose oxidase content¹²⁹. A similar finding has been made-in respect of the sensitivity of glucose oxidase to denaturation by light, a photosensitizing component responsible for the photo-oxidation of the enzyme being partially characterized in this study¹³⁰. Of course, the influence of these factors on the antibacterial activity depends on the degree of exposure of honey samples to heat and light before they are assayed, but it is likely that much of the variation seen in the antibacterial activity of honeys reflects the history of those honeys. The level of antibacterial activity in a honey has for a long time been taken as an indication of whether or not a honey has been subjected to heating in its processing, although with the realization that it depends on other factors as well, this measure is no longer recommended¹²⁹.

Bactericidal or bacteriostatic action?

Duration of bacteriostasis

Most of the reports on the antibacterial activity of honey do not allow a distinction to be made between whether a honey is killing the bacteria or whether it is just stopping the bacteria from growing. Although no growth may have been seen over the period of observation, sometimes up to four days, in the absence of other evidence this only can be taken to be a *bacteriostatic* action, even if termed a *bactericidal* action by some authors^{14,67,81}. A bactericidal action only can be concluded to have been observed in those studies where subculturing in a honey-free medium after initial exposure to honey shows no subsequent growth, which is what is recorded as bactericidal action in table 1. There may have been a bactericidal action in additional instances, but this cannot be known without the additional experimentation needed to demonstrate it.

Limited experimentation in these studies may also have left instances of bacteriostasis unobserved. In most cases a bacteriostatic action was demonstrated by lack of visible growth at the end of a period of incubation, a single observation being made at the end. It is likely, especially in the many studies with a long period of incubation, that growth would have ceased well before the observation was made: in batch culture, exhaustion of nutrient or build-up of toxic end-products can limit growth in quite a short time. In these cases a partially inhibited culture could 'catch up' an uninhibited control at this point of limited growth before the growth was observed. It is also possible that complete inhibition of growth could have gone unobserved in these studies: in other studies involving monitoring throughout incubation there is evidence of bacteria overcoming the antibacterial activity of honey after a period of inhibition^{25,52,101}. However, the period was found to be longer with higher concentrations of honey, and seven major wound-infecting species of bacteria were found to be kept in a state of complete inhibition for 8 h if the concentration of honey was increased to between 3% and 10%¹³¹.

Complete inhibition of growth maintained over a long period is obviously an important feature in controlling infections. Also of relevance is that if bacteria are kept in a state of bacteriostasis for a long period, their capacity to recover is lost¹⁰⁹. In most of the cases of complete inhibition listed in table 1, the period of study over which this was maintained was 18-24 h.

Bactericidal action of honey

Whether or not honey has a bactericidal action appears to be very much a matter of the time of exposure of the cells to the honey. A gradual decline over 24 h was seen in the number of viable cells of several species of bacteria killed by 10% honey²⁵. In another study⁸⁴ a bactericidal action was seen against *Escherichia* with 17% honey after 24 h, but 48 h was required for bactericidal action with 9% honey: with *S. aureus* 24 h was required for bactericidal action with 33% honey, 48 h was required for bactericidal action with 25% honey, and 96 h for bactericidal action with 9% honey. Another factor which contributes to this variation is differences in the susceptibility of the species being used for testing. The action of 20% honey was found to be bactericidal on only two out of six species of bacteria tested⁵². Observation of the bactericidal action of 50% honey on 12 species²⁰

revealed that Gram-positive species generally were the first to be killed, starting to die after 1 h of exposure, with complete killing after 3-24 h. Gram-negative species generally began to die after 4-6 h, complete killing taking up to 48 h. A comparison of ten species of bacteria exposed to eight different honeys at 50% concentration⁵⁷ found that the time required for a complete bactericidal action ranged from 3 to 48 h, there being four-fold differences between the honeys, and larger differences between the species. Another study⁶⁹ also showed that the time required for bactericidal action depends on the species of bacteria, and on the concentration of honey: *E. coli* with 50% honey grew for 2 h then began to decline in the number of viable cells; *S. aureus* showed a decline by 1 h, with complete killing after 4 h with 50% honey, but only partial killing by 5 h with 25% honey.

An incomplete bactericidal action in the time allowed would have been taken as no bactericidal action by some investigators who simply looked for growth after exposure: a small number of surviving cells would give this. Thus it was concluded that there was no bactericidal action on 6 species by 5-10% honey over a period of 5 h⁵⁰. Likewise it was concluded that there was no bactericidal action on *S. aureus* by 15% honey over a period of 5 h¹²⁷, and by 29% honey over a period of 36 h¹³¹. Also with 11 species exposed to 10% honey for 8 h it was concluded that the action was only bacteriostatic⁵⁴, but it was noted that on subsequent examination there was evidence of damage to the bacterial cells.

It is known that vegetative cells of bacteria will die off slowly at sub-optimal levels of water activity (a_w)⁷⁶. Even so, in full strength honey this can take up to 34 days for *Salmonella* at 18-20°C¹¹⁵, and up to 2 years at 10°C¹¹⁶. However, in another study⁹⁵ much shorter times were found to be needed (up to 3 days at room temperature). Differences in the composition of the honeys used could well account for these differences in findings: different times were found to be required for different honeys to kill a particular species within the same experiment in two studies^{95,105}. Differences in the composition of the honeys used could also account for differences in conclusions on whether the antibacterial action of honey is bacteriostatic or bactericidal. In several studies^{14,20,40,112,131} only some of the honeys tested had a bactericidal activity at the concentrations used.

It may just be a matter of longer times or higher concentrations being required for bactericidal activity to be seen: many bacteriostatic substances are bactericidal at higher concentrations¹⁰⁹. Low a_w may greatly influence the microbicidal effect of other factors⁷⁶, thus these factors may be of more consequence in honeys at high concentrations.

Although hydrogen peroxide gives bacteriostasis with *S. aureus* at 0.29 mmol/l or lower^{8,23,127}, it has been found that 29 mmol/l hydrogen peroxide is required to kill *E. coli* and *S. aureus* in 1 and 8.8 mmol/l to achieve a kill rate of 80% in 1 h with seven strains of bacteria¹¹⁴. The quantities of hydrogen peroxide that are produced in honey (discussed earlier) are unlikely to accumulate to such levels, but could be high enough in some honeys to be bactericidal over a longer period of exposure, especially with the influence of a low a_w and with potentiation by metal ions and ascorbic acid. The presence of plant-derived bactericidal factors in some honeys, helped by the low a_w may also account for some honeys being bactericidal.

Whether or not honey is bactericidal is of little practical significance however. Some of the antibiotics in common use in medical practice have only a bacteriostatic

Action¹⁰⁴. Complete bacteriostasis, maintained by regular application of honey, would be sufficient to allow the healing process to work successfully. The fairly rapid clearing up of infections that is found to occur under a dressing of honey^{11,15,32,48} may be a result of bactericidal action from prolonged exposure, or possibly be a result of the natural defence system being more successful with multiplication of bacterial cells held in check.

Stability of antibacterial activity

The instability of honey inhibine was first recognized in 1937 by Dold et al.²⁷, who found that it was destroyed by heating and by exposure to light. These observations have since been confirmed by numerous other researchers, but there have been differences in the degree of instability reported.

Sensitivity to heat

The initial report (Dold et al.²⁷) on the loss of antibacterial activity on exposure of honey to heat was of complete loss of inhibition by 17% honey after exposure of 50% honey to 100°C for 5 min, 80°C for 10 min, or 56°C for 30 min. However, this did not mean that antibacterial activity was lost completely: if the unheated honey had been of just high enough activity to inhibit growth when at 17%, not much activity would have to be lost on heating for inhibition no longer to be seen. This also applies to the similar finding of Pothmane that exposure of honey to 100°C for 5 min or 56°C for 1 h caused complete loss of inhibition by 17% honey. In later reports the researchers used a dilution series for the assay of activity. Although complete loss of inhibition in their studies still did not mean that antibacterial activity was lost completely, its reduction to a level below detestability would generally represent a loss of 80% or more, if not a complete loss. In these reports 'complete loss' was found to result from exposure of honey to: 100°C for 30 min; 100°C for 15 min³⁶; 90°C for 8 min⁴⁵; 100°C for 5 min, 90°C for 15 min, 70-80°C for 20-30 min, and 56°C for 60 min⁴⁰; 80°C for 15 min¹⁰⁸; 80°C for 30 min⁵²; 60°C for 15 min¹³²; and from use of 'heated honey' (no details given)³⁶. An almost complete loss was found on heating honey for 100°C for 10 min¹⁷. In another report²⁰ the activity was not lost completely after exposure of honey to 100°C for 15 min, but was reduced to the same level as that of artificial honey, indicating that all activity other than that due to osmolarity had been destroyed. A similar finding was made with honey boiled for 10 min⁸¹. Others also have found that only part of the antibacterial activity is destroyed by heating honey. Exposure of honey to 100°C for 10 min caused complete loss of activity against seven species of bacteria, but only partial loss of activity against *Bacillus pumilus* and a strain of *Streptomyces*, and no loss of activity against *Bacillus subtilis* and *Sarcina lutea*. Another report⁴² about half of the activity against *B. subtilis* was found to be heat-stable. Heating honey at 56°C for 30 min caused a loss of activity that was greater against some species than against others'. The presence of both heat-stable and heat-sensitive factors has been reported by others also^{8,16,17,24,52}. The retention of part of the activity reported in instances where honey has been

subjected to lesser degrees of heating probably results from there being only partial destruction of the heat-sensitive factor, rather than a heat-stable factor being responsible. The minimum inhibitory concentration of honey was found to increase from 4% to 8% after exposure of honey to 46°C for 8 h, to 12% after exposure to 52°C for 8 h, and to 16% after exposure of honey to 55°C for 8 h¹³². Also reported¹⁰⁸ was complete loss of activity after exposure to more than 65°C for less than 4 h, a heavy but not complete loss after exposure to 56°C for 24 h, but no loss after exposure to 40°C for 96 h. In another report⁵¹ there was complete loss of activity after exposure to 100°C for 30 min, but no loss after exposure to 56°C for 30 min. Also reported⁸⁴ was complete loss of activity after exposure to 100°C for 5 min, but only partial loss after exposure to 60°C for 1 h.

The stability of the antibacterial activity in heated honey has been found to depend on the pH, activity being more rapidly lost at low pH¹⁴⁵.

There are some large differences in the findings on the stability of the antibacterial activity of honey at lower temperatures, but generally the conclusion has been that it is stable below 40°C. No decrease in antibacterial activity was seen in 20 honeys held at 40°C for 96 h³¹, as in the case mentioned above¹⁰⁸, nor in honey held at 37°C for 24 h²⁸. This is to be expected when it is borne in mind that the temperature in the beehive where honey can spend quite a long time is around 34°C. It may not be as stable at this temperature when diluted: the rate of production of hydrogen peroxide drops off with time¹²⁷, and the amount of hydrogen peroxide present after 16 h was found to be much lower than that present after the first hour⁸. Others have also reported that honey is less stable when diluted^{3,16,17,27,28,42}. This could be a consequence of the build-up of gluconic acid, or of damage to the glucose oxidase from free radicals generated from hydrogen peroxide as discussed earlier. The latter suggestion is supported by the finding with the isolated enzyme that addition of a high level of hydrogen peroxide inactivated it after about 30 min⁹⁸. However, it has been reported that 50% honey held at room temperature for 100 h does not lose its antibacterial activity^{42,127}. There are several indications of the antibacterial activity of honey being very stable at room temperature. In one study¹²¹ of a large number of honeys it was noted that 43 of the 85 honeys with high antibacterial activity were 9 months to 1 year old, and some were 2 years old. It was stated that the antibacterial activity could be retained on long storage in the laboratory if the honey was kept away from light and high temperatures. Another study⁴ of a large number of honeys also found high activity in old samples of honey, some up to 5 years old, there being no correlation between the activity and the age of the honey. A study of 18 honeys found some of the most active to be 2-3 years old^{16,17}. Storage of honey without deterioration of its antibacterial activity for several months at 20°C⁴², also for 2 years at 25-30°C¹²², has been reported. Little or no loss of activity was found in honeys stored for 1 year at room temperature in closed containers, but loss of activity was noted in samples that had been opened frequently⁶⁶. On the other hand almost complete loss of activity was found on storage of honeys for 18 months at 4°C in the dark^{30,94}. Also reported⁸ has been a loss of 15-16% in 3 months and 24-27% in 6 months storage at 20-25°C. These differences in findings could be the result of there being differences in the stability of various honeys. Marked differences in the proportions of activity lost

at each temperature tested have been reported by several authors^{8,31,42,54,61}. A 70-fold difference was found between honeys in the half-life of the peroxide-accumulating system of various honeys¹²⁹. This appeared to be related to the floral source of the honey, prompting the suggestion that plant-derived substances influenced the stability of the glucose oxidase. As the half-life of the enzyme isolated from honey is approximately 5 min at 50°C, and the half-life of the peroxide-accumulating system was found to range from 2.8 to 6.1 h at 55°C¹²⁹, it appears that this influence is a stabilizing one. The half-life of the peroxide-accumulating system was determined for higher temperatures as well. In addition to their own measurements, White and Subers¹²⁹ estimated the half-life of the antibacterial activity in honey from the data published by others on its decline on heating. They found that at 65°C it ranged from 36 s to 4.5 min, and at 70°C, with a larger number of samples, it ranged from a few seconds to 1 hour. Their estimates from others' data were 4.5 h at 62.8°C, and 10 h at 57°C.

Sensitivity to light

It has been known since some of the earliest work on the antibacterial properties of honey that the activity is unstable in light. Dold *et al.* in 1937²⁷ reported that honey lost its ability to inhibit bacterial growth (tested in a 17% solution) after exposing a thin film of it to sunlight. Others have since confirmed this observation. Exposure of honey in a layer 1-2 mm thick to sunlight for 15 min was found to result in complete loss of non-osmotic activity¹³⁰. When not spread out in a thin layer it has not been found to be so sensitive: almost complete loss of activity after 18 days in direct sunlight⁶¹, gradual disappearance of activity when exposed to direct sunlight but not with diffuse daylight⁴⁰, and a significant reduction in activity in honey samples stored for 3-6 months on open shelves (more than twice that lost in the same samples stored in a dark cupboard)⁸ have been reported. No loss of activity was found, however, when a thin film of honey was exposed for 1 h to an ultraviolet (UV) lamp (254 nm)¹⁷.

A large loss of activity was found in honey left for 8 months on a window-sill on the sunny side of the building if stored in 1 or 2.5 litre jars made from clear polystyrene, but not if stored in jars made of white or ivory polyethylene with low transmission of light of wavelength below 400 nm¹²². Glass jars coated with a film to absorb UV light were only partially successful in this study in preventing the loss of activity, indicating the necessity to protect from light of wavelengths up to 400 nm¹²². Similar findings were made in another study: honey stored for 5-7 months by the window in the sun lost about half of its activity if kept in UV-absorbing glass jars, but kept all its activity if kept in jars made of dark glass³¹. This protection by absorption of light can occur within the honey itself, as is seen with the greater stability of bulk quantities compared with thin films. Dark-coloured honey was found to be more light-stable than light-coloured honey⁸, presumably because it s less light into the bulk of the honey. However, the sensitivity to light has been observed to depend on the floral source of the honey³⁵: in a 500 g jar kept in sunlight, some floral types of honey were found to lose their activity completely in only 48 h³⁵, and a reduction of up to 67% in the production of hydrogen peroxide

The components responsible for the antibacterial activity of honey

Acidity

The pH of honey is low enough to slow down or prevent the growth of many species of bacteria, but this acidity may be neutralized if honey is diluted with buffering solutions such as body fluids.

Osmolarity

The high sugar content of honey makes the water unavailable for micro-organisms: no bacteria or fungi can grow in fully ripened honey, but the more diluted honey becomes, the more species can grow in it.

Hydrogen peroxide

The glucose oxidase enzyme activated by dilutions of honey generates hydrogen peroxide which generally is the major antibacterial factor in honey. This enzyme is inactivated by heating honey, and by exposure to light in some honeys which contain a sensitizing factor. Some honeys also contain substances which destroy the hydrogen peroxide generated by the enzyme.

Other components

Honeys from some floral sources contain various antibacterial substances, presumably produced by certain species of plants, which in some case can account for a large part of the antibacterial activity of honey.

was found 4-5 cm in from the glass after only 6 h³⁴. Difference in floral source could account for the finding in an early study that honey was nearly insensitive to diffuse daylight, it standing for 20 months in the laboratory.

Although hydrogen peroxide is degraded by exposure to light, this cannot account for the sensitivity of the antibacterial activity of honey to light as there is so little hydrogen peroxide present in full-strength honey. It has been found that in fact it is the glucose oxidase, that generates the hydrogen peroxide, that is sensitive to light¹³⁰. The marked disagreement seen in observations on the stability of the antibacterial activity of honey to light can be explained by the finding that a photosensitizer is necessary for the photo-oxidation of the enzyme, and that it occurs in different amounts in different honeys¹³⁰. Another variable could be the dependence of the light-sensitivity on the pH, which can vary between 3.2 and 4.5¹²⁴ in different honeys. The sensitivity of the glucose oxidase activity to light was found to be minimal at pH 8 but to increase sharply from pH 5 downwards¹³⁰. It is not known whether it is the photosensitizer or the enzyme that is influenced by the pH.

The hydrogen peroxide accumulating system in honey was found to be strongly influenced by diffuse daylight and the light from fluorescent tubes¹²⁸, which is more detrimental than the light from incandescent bulbs³⁴. Nearly half of the enzyme activity was found to be lost in a 10 g sample of honey left for 2 h on the laboratory bench in a 50 ml beaker¹³⁰. The sensitivity of the photosensitizer/enzyme was found, using various lamps and filters, to be greatest to light in the wavelength band 425-525 nm¹³⁰.

There have also been reports of non-peroxide antibacterial factors being light-sensitive^{59,118}.

Conclusion

It has been shown that the potency of the antibacterial activity can vary very markedly. The number of variable factors involved makes it impossible to predict with any certainty that a particular honey will have a high antibacterial activity. Because of this, honeys purveyed for therapeutic use should be assayed for their antibacterial activity as a form of quality assurance.

Consideration should also be given to the way that honey is processed if it is intended for sale as an antibacterial product (fig. 6). Honey is often pasteurized, at a temperature of 70-75°C, to destroy yeasts that can spoil a honey with a high water content, or to dissolve sugar crystals that could initiate granulation in a liquid honey. In view of the short half-life of the antibacterial activity at pasteurization temperatures, it is clear that pasteurization of honey is undesirable if the honey is to be used as an antiseptic. It would also be advisable to keep any other warming of the honey during processing to a minimum, and to store it at cool temperatures.

Another consideration regarding processing for marketing liquid honey should be the likely effect on antibacterial activity when honey is filtered to remove pollen and other particles which can initiate granulation, since it has been found that glucose oxidase is adsorbed on to the asbestos filter pads in Seitz filtration. It remains to be determined whether the enzyme is removed by absorption on to filtration aids used in the clarifying of liquid honeys, but some of the filtration aids used are very effective in removing proteins from other products.

As a further precaution against possible loss of antibacterial activity, honeys with high activity should not be blended with honey of low activity: a honey with low activity could well have components present that destroy antibacterial activity.

Loss of antibacterial activity on exposure to light is another important consideration. Because there is little certainty about which floral sources give honeys that are sensitive to light, and because some can be very sensitive, it is important that honey intended for therapeutic use be protected from light to prevent possible reduction of its antibacterial activity. For retail sale it could well be packaged in brown glass containers like other medical products.

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FIG. 6. Manuka honey marketed as an antibacterial product.

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References

1. Aoccox, D (1962) The effect of catalase on the inhibine and peroxide values of various honeys. *Journal of Apicultural Research* 1: 38-40.
2. AGNER, K (1963) Studies on myeloperoxidase activity. 1. Spectrophotometry of the MPO-H₂O₂ compound. *Acta Chemica Scandinavica* 17 (Suppl. 1): 5332-5338.
3. AGOSTINO BARBARO, A D; ROSA, C LA; ZANELLI, C (1961) Attività antibatterica di mieli Siciliani. *Quaderni della Nutrizione* 21(1/2): 30-44.
4. ALLEN, K L; MOLAN, P C; REID, G M (1991) A survey of the antibacterial activity of some New Zealand honeys. *Journal of Pharmacy and Pharmacology* 43(12): 817-822.
5. AMOR, D M (1978) *Composition, properties and uses of honey—a literature survey*. The British Food Manufacturing Industries Research Association; Leatherhead, UK; Scientific and Technical Surveys No.108; 84 pp (confidential).
6. ARISTOTLE (350 BC) Volume IV. *Historia animalium*. In Smith, J A; Ross, W D (eds) *The works of Aristotle*. Oxford University; Oxford, UK (translated by Thompson, D'A W, 1910).
7. BAIRD-PARKER, A C; HOLBROOK, R (1971) The inhibition and destruction of cocci. In Hugo, W R (ed) *Inhibition and destruction of the microbial cell*. Academic Press; London, UK; pp 369-397.

8. BOGDANOV, S (1984) Characterisation of antibacterial substances in honey. *Lebensmittel-Wissenschaft und Technologie* 17(2): 74-76.
9. BOGDANOV, S (1989) Determination of pinocembrin in honey using HPLC. *Journal of Apicultural Research* 28(1): 55-57.
10. BOGDANOV, S; RIEDER, K; ROEGG, M (1987) Neue Qualitätskriterien bei Honiguntersuchungen. *Apidologie* 18(3): 267-278.
11. BRANKI, FJ (1981) Surgery in western Kenya. *Annals of the Royal College of Surgeons of England* 63: 348-352.
12. BREED, R S; MURRAY, E G D; HIRCHENS, A P (1948) *Bergey's manual of determinative bacteriology*. Williams & Wilkins; Baltimore, USA; 1529 pp (6th edition).
13. BUCHANAN, R E; GIBBONS, N E (1974) *Bergey's manual of determinative bacteriology*. Williams & Wilkins; Baltimore, USA; 1246 pp (8th edition).
14. BUCHNER, R (1966) Vergleichende Untersuchungen über die antibakterielle Wirkung von Birten- und Honigtauhonigen. *Südwestdeutscher Linker* 18: 240-241.
15. CAVANAGH, D; BEAZLEY, J; OSTAPOWICZ, F (1970) Radical operation for carcinoma of the vulva. A new approach to wound healing. *The Journal of Obstetrics and Gynaecology of the British Commonwealth* 77(11): 1037-1040.
16. CHAMBONNAUD, J P (1966) Etude du pouvoir anti-bactérien des miels par une technique de diffusion en gelose. *Bulletin apicole* 9(1): 83-98.
17. CHAMBONNAUD, J P (1968) Contribution à la recherche des antibiotiques dans le miel. *Bulletin apicole* 11(2): 133-200.
18. CHIRIFE, J; SCARMATO, G; HERSZAGE, L (1982) Scientific basis for use of granulated sugar in treatment of infected wounds. *The Lancet*: 560-561.
19. CHRISTIAN, I H B; WALTHO, J A (1964) The composition of *Staphylococcus aureus* in relation to the water activity of the growth medium. *Journal of General Microbiology* 35: 205-218.
20. CHRISTOV [KRISTOV], G; MLADENOV, S (1961) Propriétés antimicrobiennes du miel. *Comptes rendus de l'Académie bulgare des Sciences* 14(3): 303-306.
21. CHWASTEK, M (1966) Jakosc miodowyszczelich handlowych na podstawie oznaczania ich skladnikiem niecukrowcowych. czesc II. Zawartosc inhibiny w miodach krajowych. *Roczniki Panstwowego Zakladu Higieny* 17(1): 41-48.
22. COHEN, G; HOCHSTEIN, P (1962) Glutathione peroxidase: the major pathway for the detoxification of hydrogen peroxide in erythrocytes. *American Chemical Society. 141st, Meeting. Division of Biological Chemistry. p 58c* (abstract no. 137).
23. COULTHARD, C E; MICHAELS, R; SHORT, W F; SYKES, G; SKRIMSHIRE, G E H; STANDFAST, A F B; BIRKINSHAW, J H; RAISTRICK, H (1945) Notatin: an anti-bacterial glucose-aerodehydrogenase from *Penicillium notatum* Westling and *Penicillium resticulosum* sp. nov. *Biochemical Journal* 39: 24-36.
24. DAGHIE, V; CIRNU, CIOCA, V (1971) Contribution to the study of the bacteriostatic and bactericidal action of honey produced by *Physokermes* sp. in the area of coniferous trees. *Proceedings of the XXIIIrd International Apicultural Congress, Moscow*. Apimondia Publishing House; Bucharest, Romania; pp 593-594.
25. DAGHIE, V; CIRNU, I; CIOCA, V (1973) Contributii privind actiunea bactericide si bacteriostatice a mierii de lecaniide (*Physokermes* sp.) din zona coniferelor. *Apicultura* 26(2): 13-16.
26. DAVIS, B D; DUBELCCO, R; EISEN, H N; GINSBERG, H S; WOOD, W B (1973) *Microbiology*. Harper and Row; Hagerstown, Maryland, USA; 1562 pp (2nd edition).
27. DUO, H; DU, D H; DZIAO, S T (1937) Nachweis antibakterieller, hitze- und lichtempfindlicher Hemmungsstoffe Inhibine im Naturhonig Blütenhonig. *Zeitschrift für Hygiene und Infektionskrankheiten* 120: 155-167.
28. DOT, O, H; KNAPP, T (1949) Über inhibierende und modifizierende Wirkungen des Honigs auf Diphtheriebacillen und seine Brauchbarkeit zur Bekämpfung des Diphtheriebacillentragerums. *Zeitschrift für Hygiene und Infektionskrankheiten* 130: 323-334.
29. DUO, H; WITZENHAUSEN, R (1955) Ein Verfahren zur Beurteilung der örtlichen inhibitorischen (keimvermehrungshemmenden) Wirkung von Honigsorten verschiedener Herkunft. *Zeitschrift für Hygiene und Infektionskrankheiten* 141: 333-337.
30. DOLEZAL, M; DOLEZAL, M; MEDRELA-KUDER, E (1988) Research on inhibine effect of herb-honey. *Acta Biologica Cracoviensis Series Botanica* 30: 9-16.
31. DUISBERG, H; WARNECKE, B (1959) Erhitzungs- und Lichteinfluss auf Fermente und Inhibine des Honigs. *Zeitschrift für Lebensmitteluntersuchung und -Forschung* 111: 111-119.

32. DRONGLERT, 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.
33. D U S T M A N N, J H (1971) Über die Katalaseaktivität in Bienenhonig aus der Tracht der Heidekrautgewächse (Ericaceae). *Zeitschrift für Lebensmitteluntersuchung und -Forschung* 145: 294-295.
34. D U S T M A N N, J H (1972) Über den Einfluß des Lichtes auf den Peroxid-Wert (Inhibin) des Honigs. *Zeitschrift für Lebensmitteluntersuchung und -Forschung* 148(5): 263-268.
35. D U S T M A N N, J H (1979) Antibacterial effect of honey. *Apiacta* 14(1): 7-11.
36. D U S T M A N N, J (1987) Effect of honey on the cariogenic bacterium *Streptococcus mutans*. *Proceedings of the XXXIst International Apicultural Congress of Apimondia, Warsaw, Poland*. Apimondia Publishing House; Bucharest, Romania; pp 459-461.
37. E F F E M, S E E (1988) Clinical observations on the wound healing properties of honey. *British Journal of Surgery* 75: 679-681.
38. F L O R I S, P R O T A, R (1989) Sul miele amaro di Sardegna. *Apicoltore Moderno* 80(2): 55-67.
39. F O T I D A R, M R; F O T I D A R, S N (1945) 'Lotus' honey. *Indian Bee Journal* 7: 102.
40. F R A N C O, M; S A R T O R I, L (1940) Sull'azione antibatterica del miele. *Annali d'Igiene* 50: 216-227 (abstracted in *The Lancet* i: 1184 (1940)).
41. G A U H E, A (1941) Über ein glukoseoxydierendes Enzym in der Pharynxdrüse der Honigbiene. *Zeitschrift für Vergleichende Physiologie* 28(3): 211-253.
42. G O N N E T, M; L A V I E, P (1960) Influence du chauffage sur le facteur antibiotique present dans les miels. *Annales de l'Abeille (Paris)* 3(4): 349-364.
43. G R E C E A N U, A; E N C I U, V (1976) Observations on the antibiotic effects of propolis, pollen and honey. *2nd International Symposium on Apitherapy, Bucharest*. Apimondia Publishing House; Bucharest, Romania; pp 177-179.
44. G R O C H O W S K I, J; B I L I N S K A, M (1987) Biological activity of pollen, bee bread and honey relative to selected bacterial strains. *Proceedings of the XXXIst International Apiculture Congress of Apimondia, Warsaw, Poland*. Apimondia Publishing House; Bucharest, Romania; pp 462.
45. G R Y U N E R, V S; A R I N K I N A, A I (1970) (Carbohydrates content, enzymatic and antimicrobial activity of honey.] *Izvestiya Vysshikh Uchebnykh Zavedenii Pishchevaya Tekhnologiya* 1970(6): 28-31 (original in Russian).
46. G U N T H E R, R T (1934) *The Greek herbal of Dioscorides*. Hefner; New York, USA; 701 pp (translated by Goodyear, J, 1655).
47. H A F F E J E E, I E; M O O S A, A (1985) Honey in the treatment of infantile gastroenteritis. *British Medical Journal* 290: 1866-1867.
48. H A M D Y, M H; E L - B A N B Y, M A; K H A K I F A, K I; G A D, M; H A S S A N E I N, E M (1989) The antimicrobial effect of honey in the management of septic wounds. *Proceedings of the Fourth International Conference on Apiculture in Tropical Climates, Cairo*. International Bee Research Association; London, UK; pp 61-67.
49. H A Y D A K, M H; C R A N E, E; D U I S B E R G, H; G O C H N A U E R, T A; M O R S E, R A; W H I T E, J W; P (1975) Biological properties of honey. In Crane, E (ed) *Honey: a comprehensive survey*. Heinemann; London, UK; pp 258-266.
50. H O D G S O N, M J (1989) *Investigation of the antibacterial action spectrum of some honeys*. M Sc thesis; University of Waikato, New Zealand; 83 pp.
51. I A L O M I T Z E A N U, M; D A G H I E, V; M I H A E S C U, N F (1967) Contribution to the study of the bacteriostatic and bactericidal action of honey. *Proceedings of the XXIst International Apicultural Congress, Budapest, Hungary*. Apimondia Publishing House; Bucharest, Romania; pp 209-213.
52. I A L O M I T E A N U, M; D A G H I E, V (1973) Investigations of the antibiotic qualities of honey. *Proceedings of the XXIVth International Apicultural Congress, Buenos Aires*. Apimondia Publishing House; Bucharest, Romania; pp 438-440.
53. B R A H I M, A S (1981) Antibacterial action of honey. *Proceedings of the First International Conference on Islamic Medicine*. (2nd edition) (*Bulletin of Islamic Medicine, volume 1*), Kuwait Ministry of Public Health; Kuwait; pp 363-365.
54. J A M E S, O B O; S E G R E E, W; V E N T U R A, A K (1972) Some antibacterial properties of Jamaican honey. *West Indian Medical Journal* 21(7): 7-17.
55. J A R V I S, D C (1961) *Folk medicine: a doctor's guide to good health*. Pan Books; London, UK; 184 pp.

56. JEDDAR, A; KHARSANY, A; RAMSAROOP, U G; BHAMJEE, A; HAFJEJEE, I E; 1v1oosA, A (1985) The antibacterial action of honey. An *in vitro* study. *South African Medical Journal* 67: 257-258.
57. KHRISTOV, G; MLADENOV, S (1961) Honey in surgical practice: the antibacterial properties of honey. *Khirurgiya (Moscow)* 14(10): 937-946 (original in Bulgarian).
58. KLEBANOFF, S J (1980) Myeloperoxidase-mediated cytotoxic systems. In Sbarra, A 1; Strauss, R R (eds) *The reticuloendothelial system. A comprehensive treatise. Volume 2. Biochemistry and metabolism*. Plenum Press; New York, USA; pp 270-308.
59. LAME, P (1963) Sur l'identification des substances antibacteriennes presentes dans le miel. *Comptes Rendus Academie des Sciences, Paris* 256: 1858-1860.
60. LEISTNER, L; RODEL, W (1975) The significance of water activity for micro-organisms in meats. In Duckworth, R B (ed) *Water relations of foods*, Academic Press; London, UK; pp 309-323.
61. LINDNER, K E (1962) Ein Beitrag zur Frage der antimikrobiellen Wirkung der Naturhonige. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene* 115(7): 720-736.
62. LOWBURY, E J L; AYLIFF, G A J (1974) *Drug resistance in antimicrobial therapy*. Thomas; Springfield, Illinois, USA; 211 pp.
63. MAJNO, G (1975) *The healing hand. Man and wound in the ancient world*, Harvard University Press; Cambridge, Massachusetts, USA; 571 pp.
64. MAURIZIO, A (1962) From the raw material to the finished product: honey. *Bee World* 43: 66-81.
65. McCui.Loch, E C (1945) *Disinfection and sterilization*. Henry Kimpton; London, UK; 472 pp (2nd edition).
66. McGARRY, J P (1961) The effect of aging on the inhibitory substance in various honeys for bacteria. *Bee World* 42(2): 226-229.
67. MEIER, K E; FREITAG, G (1955) Ober die antibiotischen Eigenschaften von Sacchariden und Bienenhonig. *Zeitschrift für Hygiene und Infektionskrankheiten* 141: 326-332.
68. MILLER, T E (1969) Killing and lysis of Gram-negative bacteria through the synergistic effect of hydrogen peroxide, ascorbic acid, and lysozyme. *Journal of Bacteriology* 98(3): 949-955.
69. MISHREF, A; MAGDA, S A; GHAZI, I M (1989) The effect of feeding medicinal plant extracts to honeybee colonies on the antimicrobial activity of the honey produced. *Proceedings of the Fourth International Conference on Apiculture in Tropical Climates, Cairo*. International Bee Research Association; London, UK; pp 80-86.
70. MIZRAHI, A; DORON, R (1982) Antimicrobial effects of hive products. *Israel Journal of Medical Sciences* 18(5): 23.
71. MOHRIG, W; MESSNER, I (1968) Lysozym als antibakterielles Agens im Bienenhonig und Bienengift. *Acta Biologica et Medica Germanica* 21: 85-95.
72. MOLAN, P C; RUSSELL, K M (1988) Non-peroxide antibacterial activity in some New Zealand honeys. *Journal of Apicultural Research* 27(1): 62-67.
73. MOLAN, P C; SMITH, I M; REID, G M (1988) A comparison of the antibacterial activity of some New Zealand honeys. *Journal of Apicultural Research* 27(4): 252-256.
74. MOLAN, P C; ALLEN, K L; TAN, S T; WILKINS, A L (1989) Identification of components responsible for the antibacterial activity of Manuka and Viper's Bugloss honeys (oral paper). *Annual Conference of the New Zealand Institute of Chemistry*, Hamilton, New Zealand; 1989 (unpublished).
75. MORSE, R A (1986) The antibiotic properties of honey. *Pan-Pacific Entomologist* 62(4): 370-371.
76. MossEL, D A A (1975) Water and micro-organisms in foods - a synthesis. In Duckworth, R B (ed) *Water relations of foods*. Academic Press; London, UK; pp 347-361.
77. NABRDALIK, M; SKARBEK, R (1974) [Inhibitory properties of bee's honey.] *Medycyna Weterynaryjna* 30(11): 669 (original in Polish).
78. NESTER, E W; ROBERTS, C E; PEARSALL, N N; MCCARTHY, B J (1978) *Microbiology*. Holt, Rinehart and Winston; New York, USA; 769 pp (2nd edition).
79. OBASEIKI-EBOR, E E; AFONYA, T C A; ONYEKWELL, A O (1983) Preliminary report on the antimicrobial activity of honey distillate. *Journal of Pharmacy and Pharmacology* 35(11): 748-749.
80. OBASEIKI-EBOR, E E; AFONYA, T C A (1984) In vitro evaluation of the anticandidiasis activity of honey distillate (HY-1) compared with that of some antimycotic agents. *Journal of Pharmacy and Pharmacology* 36: 283-284.

81. PLACHY, E (1944) Studie Ober die bakterizide Wirkung des Naturhonigs (Bluten- und Blatthonig) aus verschiedenen Hbhenlagen sowie einige Untersuchungen Ober die Eigenschaft der antibakteriellen Hemmungstoffe (Inhibine) im Naturhonig. *Zentralblatt far Bakteriologie* 100: 401-419.
82. POPEŠKOVIC, D; ĐAKIĆ, M; BUNCIĆ, S; RUŽIĆ, P (1983) A further investigation on the antimicrobial properties of honey. *Proceedings of the XXIXth International Congress of Apiculture, Budapest Hungary*. Apimondia Publishing House; Bucharest, Romania; pp 415-417.
83. POTHMANN, F-J (1950) Der Einfluß von Naturhonig auf das Wachstum der Tb.-Bakterien. *Zeitschrift fur Hygiene und Infektionskrankheiten* 130: 468-484.
84. PRICA, M (1938) Ober die bactericide Wirkung des Naturhonigs. *Zeitschrift fur Hygiene und Infektionskrankheiten* 120: 437-443.
85. RADWAN, S S; EL-ESSAWY, A A; SARHAN, M M (1984) Experimental evidence for the occurrence in honey of specific substances active against microorganisms. *Zentralblatt fur Mikrobiologie* 139(4): 249-255.
86. RANSOMS, H M (1937) *The sacred bee in ancient times and folklore*. George Allen and Unwin; London, UK; 308 pp.
87. REVATHY, V; BANERJI, S A (1980) A preliminary study of antibacterial properties of Indian honey. *Indian Journal of Biochemistry and Biophysics* 17 (supplement no. 242): 62.
88. RizVANov, K; BizHEv, B (1962) [Investigation of the antibacterial and antifungal properties of honey.] *Nauchni trudove* 11: 433-443 (original in Bulgarian).
89. Roos, D; BALM, A J M (1980) The oxidative metabolism of monocytes. In Barra, A 1; Strauss, R R (ed) *The reticuloendothelial system. A comprehensive treatise. Volume 2. Biochemistry and metabolism*. Plenum Press; New York, USA; pp 189-229.
90. 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.
91. RIEGG, M; BLANC, B (1981) The water activity of honey and related sugar solutions. *Lebensmittel-Wissenschaft und Technologie* 14: 1-6.
92. RUSSELL, K M (1983) *The antibacterial properties of honey*. M Sc thesis; University of Waikato; New Zealand; 147 pp.
93. RUSSELL, K M; MOLAN, P C; WILKINS, A L; HOLLAND, P T (1988) Identification of some antibacterial constituents of New Zealand manuka honey. *Journal of Agricultural and Food Chemistry* 38: 10-13.
94. RYCHLIK, M; DOLEZAL, M (1961) Wlasciwosci inhibinowe niektarych miodow Polskich. *Pszczelnia Zeszyty Naukowe* 5(2): 53-64.
95. SACKETI, W G (1919) Honey as a carrier of intestinal diseases. *Bulletin of the Colorado State University Agricultural Experimental Station* No, 252: 18 pp.
96. SCHADE, J E; MARSH, G L; ECKERT, J E (1958) Diastase activity and hydroxy-methylfurfural in honey and their usefulness in detecting heat alteration. *Food Research* 23: 446-463.
97. SCHEPARTZ, A I (1966) Honey catalase: occurrence and some kinetic properties. *Journal of Apicultural Research* 5(3): 167-176.
98. SCHEPARTZ, A I (1966) The glucose-oxidase of honey. IV. Some addition observations. *Biochimica et Biophysica Acta* 118: 637-640.
99. SCHEPARTZ, A I; SUMS, M H (1964) The glucose-oxidase of honey. I. Purification and some general properties of the enzyme. *Biochimica et Biophysica Acta* 85: 228-237.
100. SCHEPARTZ, A I; SUBERS, M H (1966) Catalase in honey. *Journal of Apicultural Research* 5(1): 37-43.
101. SCHULER, R; VOGEL, R (1956) Wirkstoffe des Bienehonigs. *Arzneimittel Forschung* 6: 661-663.
102. Scan, W I (1957) Water relations of food spoilage microorganisms. *Advances in Food Research* 7: 83-127.
103. SEDOVA, N N; USMANOV, M F (1973) [Antimicrobial properties of some types of honey from Uzbekistan.] *Voprosy Pitaniya* 32(2): 84-85 (original in Russian).
104. SHANSON, D C (1989) *Microbiology in clinical practice*. Wright; London, UK; 657 pp (2nd edition).
105. SKRYPNIK, E I; KHOROCSKII, L N (1974) [Persistence of tuberculosis in honeys]. *Pchelovodstvo* 94(5): 41 (original in Russian).
106. SMITH, M R; MCCAUGHEY, W F; KEMMERER, A R (1969) Biological effects of honey. *Journal of Apiculture! Research* 8(2): 99-110.
107. SHINSON, E E; SUBERS, M H; PETTY, J; WHITE, I W (1960) The composition of honey. V. Separation and identification of the organic acids. *Archives of Biochemistry and Biophysics* 89: 6-12.

108. Stomro-Stitz, J; KOMINOS, S D (1960) Ober bakteriostatische Wirkung des honigs. *Zeitschrift für Lebensmitteluntersuchung und -Forschung* 113: 304-309.
109. SYKES, G (1965) *Disinfection and sterilization*. Soon; London, UK; 486 pp (2nd edition).
110. TAN, S T (1989) *A chemical investigation of some New Zealand honeys*. D Phil thesis; University of Waikato; New Zealand; 201 pp.
111. THIMANN, K V (1963) *The life of bacteria*. Macmillan; New York; 909 pp (2nd edition).
112. TOMLINSON, 1T; WILLIAMS, S C (1985) Antibiotic properties of honey produced by the domestic honey bee *Apis mellifera* (Hymenoptera: Apidae). *Pan-Pacific Entomologist* 61(4): 346-347.
113. Tom, G; LEMBERKOVICS, E; KUTASI-SZABO, (1987) The volatile components of some Hungarian honeys and their antimicrobial effects. *American Bee Journal* 127(7): 496-497.
114. TURNER, F 1 (1983) Hydrogen peroxide and other oxidant disinfectants. In Block, S S (ed) *Disinfection, sterilization and preservation*. Lea and Febiger; Philadelphia, USA; pp 240-250 (3rd edition).
115. TYSSET, C; DURAND, C (1973) De la survie de quelques germes a Gram négatif, non sporulés, dans le miel du commerce. *Bulletin de l'Académie Vétérinaire de France* 46(4): 191-196.
116. TYSSET, C; DURAND, C (1976) De la survie de quelques entérobactéries dans le miel stocké dans une enceinte réfrigérée +10° C. *Bulletin de l'Académie Vétérinaire de France* 49(4): 417-422.
117. TYSSET, C; ROUSSEAU, M; DURAND, C (1980) Microbism and wholesomeness of commercial honey. *Apiacta* 15(2): 51-60.
118. VERGE, J (1951) L'activité antibactérienne de la propolis du miel et de la gelée royale. *Apiculture* 95(6: Section scientifique): 13-20.
119. WADI, M; AL-AMIN, H; FAROUQ, A; KASHEF, H; KHALED, S A (1987) Sudanese bee honey in the treatment of suppurated wounds. *Arab Medico* 3: 16-18.
120. WAITES, W M; BOUSS, C E; KING, N R; DAVIES, A M C (1979) The effect of transitional metal ions on the resistance of bacterial spores to hydrogen peroxide and to heat. *Journal of General Microbiology* 112: 225-233.
121. WARNECKE, 8; DUISBERG, H (1958) Die bakteriostatische (inhibitorische) Wirkung des Honigs. *Zeitschrift für Lebensmitteluntersuchung und -Forschung* 107: 340-344.
122. WARNECKE, B; DUISBERG, H (1964) Die Erhaltung der Honiginhibine durch Ausschaltung des UV-Lichtes. *Zeitschrift für Lebensmitteluntersuchung und -Forschung* 124: 265-270.
123. WELLFORD, T E T; EADIE, T; LLEWELLYN, G C (1978) Evaluating the inhibitory action of honey on fungal growth, sporulation, and aflatoxin production. *Zeitschrift für Lebensmitteluntersuchung und -Forschung* 166(5): 280-283.
124. WHITE, J W (1975) Composition of honey. In Crane, E (ed) *Honey: a comprehensive survey*. Heinemann; London, UK; pp 157-206.
125. WHITE, J W (1975) Physical characteristics of honey. In Crane, E (ed) *Honey: a comprehensive survey*. Heinemann; London, UK; pp 207-239.
126. WHITE, J W; SUBERS, M H; SCHEPARTZ, A I (1962) The identification of inihbine. *American Bee Journal* 102(11): 430-431.
127. WHITE, J W; SUBERS, M H; SCHEPARTZ, A I (1963) The identification of inihbine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochimica et Biophysica Acta* 73: 57-70.
128. WHITE, J W; SUBERS, M H (1963) Studies on honey Inhibine. 2. A chemical assay. *Journal of Apicultural Research* 2(2): 93-100.
129. WHITE, J W; SUBERS, M H (1964) Studies on honey Inhibine. 3. Effect of heat. *Journal of Apicultural Research* 3(1): 45-50.
130. WHITE, J W; SUBERS, M H (1964) Studies on honey Inhibine. 4. Destruction of the peroxide accumulation system by light. *Journal of Food Science* 29(6): 819-828.
131. WHIN D J (1991) *A comparative study of the antibacterial action spectrum of manuka honey and other honey*. M Sc thesis; University of Waikato; New Zealand; 112 pp.
132. WOOTON, M; EDWARDS, R A; ROWS, A (1978) Antibacterial properties of some Australian honeys. *Food Technology in Australia (May)*: 175-176.
733. YATSUNAMI, K; ECHI, T (1984) [Antibacterial action of honey and royal jelly.] *Honeybee Science* 5(3): 125-130 (original in Japanese).
134. YOIRISH, N (1977) *Curative properties of honey & bee venom*. New Glide Publications; San Francisco, USA; 198 pp.
135. ZUMI, A; LUTO, A (1989) Honey - a remedy rediscovered. *Journal of the Royal Society of Medicine* 82: 384-385.