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 BC[6], and Dioscorides, c AD 50[46], 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 properties[38]; in India lotus *(Nelumbium sceciosum)* honey is said to be a panacea for eye diseases[39]. 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 Witzenhausen for such comparisons[29].

Dold and Witzenhausen coined the term 'inhibine number' in 1955[29] 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 modification[96] 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 Witzenhausee 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 al[127].

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 Warnecke[31] 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 honeys[4] 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
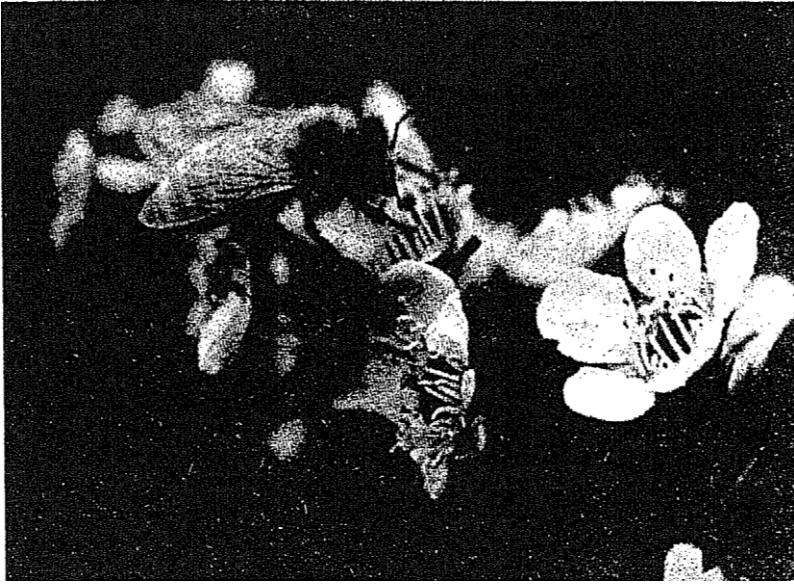
FIG. 4. Honey bee foraging on manuka flowers in New Zealand.

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 honeydew[14]. 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 a1.[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 study[4], 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
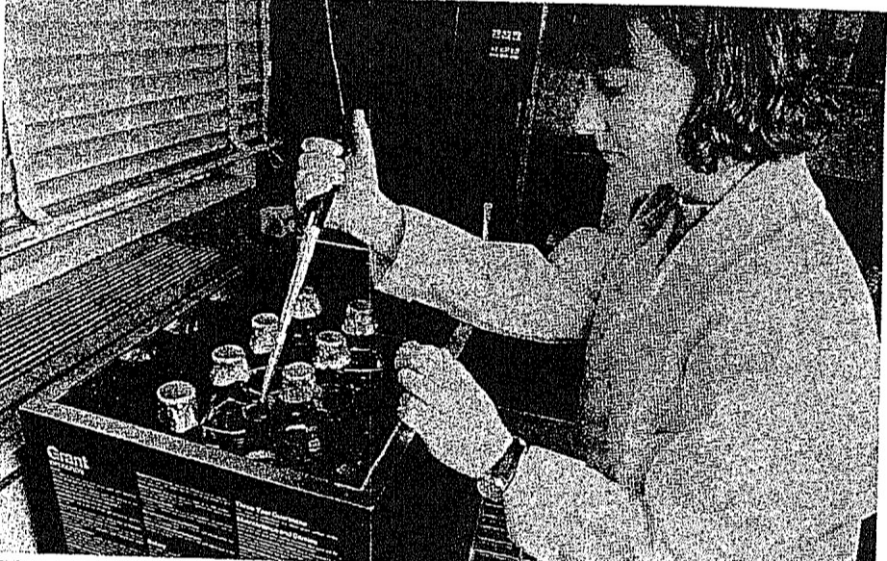
FIG. 5. Sampling broth cultures to measure the rate of bacterial growth with different concentrations of honey added.

high level of activity in one study[4], but a fairly low[94] 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 samples[72]. 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 honey[4]. 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 destruction[128].

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* a *ureus* 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 time[128].

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 honey[97], 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 nectar[33]. 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 samples[100]. 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 destruction[127]. 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 content[129]. 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 study[130]. 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 recommended[129].

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%13[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 lost[109]. 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% honey[25]. In another study[84] 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 tested[52]. Observation of the bactericidal action of 50% honey on 12 species[20]

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% concentration[57] 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 study[69] 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 h[50]. Likewise it was concluded that there was no bactericidal action on *S*. aureus by 15% honey over a period of 5 h[127], and by 29% honey over a period of 36 h[131]. Also with 11 species exposed to 10% honey for 8 h it was concluded that the action was only bacteriostatic[54], 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°C[115], and up to 2 years at 10°C[116]. However, in another study[95] 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 concentrations[109]. Low a_w may greatly influence the microbicidal effect of other factors[76], 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/1 or lower[8,23,127], it has been found that 29 mmol/1 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 bacteria[114]. 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

Action[104]. 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 mie; 100°C for 15 min[96]; 90°C for 8 11[45]; 100°C for 5 min, 90°C for 15 min, 70-80°C for 20-30 min, and 56°C for 60 min[40]; 80°C for 15 min[108]; 80°C for 30 min[52]; 60°C for 15 min[132]; and from use of 'heated honey' (no details given)[36]. An almost complete loss was found on heating honey for 100°C for 10 min[17]. In another report[20] 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 min[81]. 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 *f Streptomyces,* and no loss of activity against *Bacillus subtilis and Sarcina lutee.* another report[42] about half of the activity against *B. subtilis* was found to be eat-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 h[132]. Also reported[108] 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 report[51] 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 reported[84] 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 pF1[45].

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 h[31], as in the case mentioned above[108], nor in honey held at 37°C for 24 h[28]. 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 time[127], and the amount of hydrogen peroxide present after 16 h was found to be much lower than that present after the first hour[8]. 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 min[98]. 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 study[121] 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 study[4] 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°C[42], also for 2 years at 25-30°C[122], 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 frequently[66]. 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 reported[8] 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 honeys[129]. 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°C[129], 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 Subers[129] 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 1937[27] 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 activity[130]. 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 sunlight[61], gradual disappearance of activity when exposed to direct sunlight but not with diffuse daylight[40], 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 nm[122]. 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 nm[122]. 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 glass[31]. 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 honey[8], 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 honey[35]: in a 500 g jar kept in sunlight, some floral types of honey were found to lose their activity completely in only 48 h[35], and a reduction of up to 67% in the production of hydrogen peroxide

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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.

Osmoloarity

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.

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was found 4-5 cm in from the glass after only 6 h[34]. 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 light[130]. 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 honeys[130]. Another variable could be the dependence of the light-sensitivity on the pH, which can vary between 3.2 and 4.5[124] 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 downwards[130]. 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 tubes[128], which is more detrimental than the light from incandescent bulbs[34]. 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 beaker[130]. The sensitivity of the photosensitizer/enzyme was found, using various lamps and filters, to be greatest to light in the wavelength band 425-525 nm[130].

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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