ANTIBACTERIAL EFFECT OF HONEY*

J.H. DUSTMANN

F.R.G.

It is known that honey strongly inhibits the growth of microorganisms. Already in 1892, the Dutch scientist Van KETEL demonstrated that honey has bactericidal effects. A great number of research reports have subsequently confirmed his findings (PLACHY, DOLD, PRICA, DUISBERG, LAVIE, BUCHNER). Responsible for these antiseptic properties are both the high sugar content and, above all, the various bacteriostatic constituents. WHITE and his colleagues in the United States take the great merit of having analysed the thermolabile and light-sensitive components of this inhibitory substance (classed with the inhibine of Dold): WHITE demonstrated that the antibacterial effects of inhibine result from the accumulation of hydrogen peroxide (H_2O_2) which is enzyme-produced – by a natural glucose oxidase system in honey, and as a by–product of glucose oxidase activity in honey or sugar dilutions.

The hydrogen peroxide which is forming is partially responsible, alongside of other components, for the antibacterial effect of honey.

What relation exists between the inhibine values recorded during chemical assays (following hydrogen peroxide accumulation/minute) and the results of bioassays – inhibition of the bacterial growth not on nutrient agar plates containing honeys (produced in FRG) but in dialysed honey solutions (in test tubes)?

How great is the antibacterial effect of the enzyme prepared from the secretion of the hypopharingeal glands? Which is the effect of light or of other inhibitory factors on the inhibine activity?

Our investigations were precisely intended for answering these questions.

Method and technique

2.5 g of honey was diluted, using phosphate – 0.4 M and pH 6.5 – as substrate; then it was dialysed in dialysis dishes with running water, for removing sugars. Then the solution was sterilized by filtering and several dilutions were prepared in 10 test tubes. In each test tube, a drop of bacterial suspension and 0.2 ml glucose 10 M was pipetted. All tubes were kept in an incubator at 37 ^{o}C, for 14 hours. The level of dilution up to which the honey inhibine had inhibited bacterial growth was established by the turbidity of the solution (depending on the rate of bacterial growth).

The inhibine effect was assayed on the following bacteria: *Staphylococcus aureus*, *Streptococcus* spp., *Salmonella pullorum*, *S. gallinarum*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Sarcina lutea*, and *Proteus vulgaris*.

We used the White method modified by us.

Results

Leaving aside a few characteristic exceptions, the following relation was found to occur in general: when the peroxide content of honey is high, its bactericidal effect is very great. On the contrary, when the peroxide content is low, the inhibitory effect on the bacterial growth is slight or absent (Table 1).

It results that of the bacteria assayed by us *Staphylococus aureus* and *Sarcina lutea* were the most sensitive, while *Streptococcus* spp., *Salmonella* spp., *Pseudomonas*, and *Proteus* were less sensitive to the inhibitory action of inhibine.

Honeys with high inhibine values, e.g. of *Centaurea*, have antibacterial effect even in very low concentrations: starting from the initial concentration of 625 mg (the first test tube) we made several dilutions; the 1 : 128 dilution (only 2.45 mg honey/ml – from which sugars had been removed) was found to inhibit bacterial growth and even destroy them: the solution remained clear for several days.

Great peroxide accumulation and an antibacterial effect similar to that of such honeys with strong inhibitory action also be obtained by using the homogenate of one hypopharingeal gland (yellow greenish glands from honey bees of older age). As is but natural, the inhibine values depend on the age and physiological condition of bees. The highest value obtained up to now from one pair of glands was of 32.7 µg H_2O_2/g/min.

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Table 1

Inhibitory effect of various honey types on *Staphylococcus* (test-tube assay), for different peroxide values

Honey type	Peroxide accumulation µg H_2O_2/g/min	1 : 1 312.5 mg/ml	1 : 2 156.2	1 : 4 78.1	1 : 8 39.1	1 : 16 19.5	1 : 32 9.8	1 : 64 4.9	1 : 128 2.4	1 : 256 1.2
Centaurea	10.4	0	0	0	0	0	0	0	+	+
Decidous tree honeydew	7.52	0	0	0	0	0	+	+	+	+
Spruce + fir tree	4.3	0	0	0	0	0	0	+	+	+
Spruce + fir tree	2.5	0	0	0	0	0	+	+	+	+
Spruce + fir tree	2.4	0	0	0	0	0	+	+	+	+
Rape	1.5	0	0	0	0	+	+	+	+	+
Heather + mixed	1.7	0	0	0	+	+	+	+	+	+
Acacia	0.4	0	+	+	+	+	+	+	+	+
Rape	1.1	+	+	+	+	+	+	+	+	+
Glucose oxidase (1µg) (Boehringer)	>25	0	0	0	0	0	0	0	0	0
Heated glucose	0	+	+	+	+	+	+	+	+	+
Glucose 0.1 M	-	+	+	+	+	+	+	+	+	+

0 = clear solution, no bacterial growth; + = turbid solution, with bacterial growth

Comparatively we point out the effect of the glucose-oxidase obtained from yeasts, now available in the market (Boehringer, No. 154222). This enzyme, with 2.44 ng*/ml concentration, lyophilized and specifically purified has a great inhibitory effect on staphylococci.

A very high peroxide accumulation was also recorded in beebread – the pollen-honey mixture to which bees add different secretions: 2 g of bee bread produced a maximum amount of 47.6 µg H_2O_2/g/minute, with a corresponding inhibitory action on bacterial growth (*Staphylococcus*).

Which is the factor which makes honeys to differ in this respect? Theoretically, the following relation should be true: the more secretion bees add to the nectar or honeydew – hence the gland enzyme glucose oxidase, the higher the peroxide accumulation and the greater the antibacterial effect of honey. But this relation is not always true, because of various natural inhibitory factors existing in honey.

1. Firstly, catalase: it is a natural constituent of a number of honeys; it originates from pollen and from nectar; it splits the H_2O_2 produced by glucose oxidase, with water and oxigen resulting.

Especially in *Ericaceae* and *Rosaceae* honeys (*Prunus, Malus*, and other fruit trees) we found a great catalase activity, while the inhibitory effect was small as it was but natural.

In general, with great catalase activity the inhibine value is relatively low, while in honeys devoid of catalase activity (*Centaurea*, sweet chestnut, etc.) inhibine values are very light (Table 2).

Table 2

Catalase and inhibine activity (H_2O_2 accumulation) in *Ericaceae* honey (1-6) and in fruit-tree honey (7). 8-11 = control honeys

Honey	Catalase [a)]	Inhibine [b)]
1. *Calluna vulgaris*	46.1	111.8
2. *Erica tetralix*	104.0	2.7
3. *Vaccinium myrtillus*	198.0	127.5
4. *Erica cinerea*	119.0	187.2
5. *Rhododendron* spp.	241.0	15.3
6. *Erica vagans*	54.0	10.6
7. *Prunus* spp.	193.0	99.5
8. *Trifolium repens*	0	380.0
9. *Centaurea cyanus*	0	624.0
10. *Castanea sativa*	0	545.0
11. *Pinus silvestris*	0	662.5

a) Kfx10^3; b) µg H_2O_2/g honey/hour

Definition of the Kf indice: the Kf indice measuring the catalase activity is calculated as follows:

$Kf = \frac{1}{t} (1\ n\ \frac{Xo}{X}) \frac{D}{W}$, *where t = time; Xo = the substrate at to (the beginning of the reaction); X = the substrate after* t*; D = the dilution factor; W = weight of honey (in g).*

2. Vitamin C (ascorbic acid) or other reducing substances can easily destroy the peroxide (H_2O_2) produced. There may also be other unknown substances which could inactivate glucose oxidase (no investigation in this respect has been made yet).

** nanogram*

3. Another factor which can affect the peroxide value and consequently the inhibine value too, which under natural conditions in the bee colony has an importance but may become very important for the antibacterial effect of honey during its extraction, processing, and storage is the direct *light*. This matter has been investigated by WHITE and myself.

In an earlier report I have demonstrated the effect of direct sunlight and of the fluorescent light on the glucose oxidase activity, hence on the natural properties of honey. The table below shows that the light-sensitivity of honey depends on the source of the honey (See Figure 1 and Table 3).

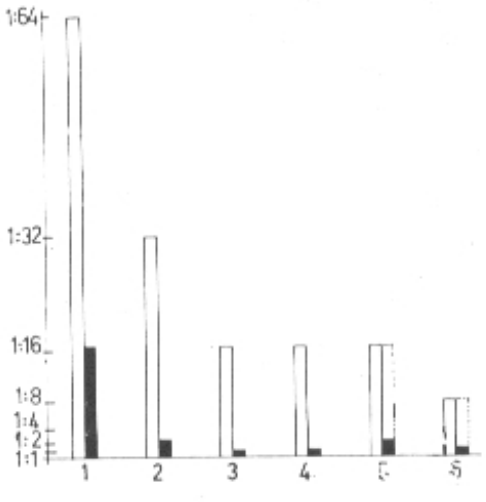

Fig. 1 – Inhibitory effect of various honeys (exposed and not to light) on Staphylococcus

❑ Full inhibitory effect of honey not exposed to light
■ Full inhibitory effect after exposure to sunlight (15 min)
❑ Reduced inhibitory effect after exposure to sunlight (15 min)

1. *Centaurea* 10.4/2.1 µg H_2O_2/g/min
2. *Centaurea* 6.1/0.9 µg H_2O_2/g/min
3. *Calluna* 2.0/1.6 µg H_2O_2/g/min
4. *Brassica* 1.5/0.2 µg H_2O_2/g/min
5. Decidous tree honeydew 7.5/7.7 µg H_2O_2/g/min
6. Spruce + fir tree 6.1/5.6 µg H_2O_2/g/min

1 : 1 - 1 : 64 = dilution

Table 3

Reduction of peroxide accumulation in various honeys after exposure of 5 mm layer to sunlight (7 x 40^4 lux) for 10 minutes

Honey	pH value	Peroxide accumulation		Reduction %
		Not exposed	Exposed	
Pine, *Pinus*[b]	4.9	418.7	113.3	1.3
Decidous tree "honeydew" [b]	5.2	378.0	368.9	2.4
Fir, *Abies*	5.1	270.0	258.9	4.1
Spruce, Picea [b]	5.0	168.5	147.1	12.7
Sweet chestnut, *Castanea* [b]	5.1	510.0	432.0	15.3
Heather, *Calluna*	4.5	132.2	103.2	22.0
Lime, *Tilia*	4.4	188.0	143.9	23.5
Dandelion, *Taraxacum*	4.3	243.7	186.2	23.6
Germander, *Teucrium*	3.9	38.5	19.9	48.3
Cornflower, *Centaurea*	4.3	624.5	308.5	50.6
Fruit-trees, *Prunus, Malus*	4.6	35.5	13.7	61.0
Rape, *Brassica*	4.6	73.3	25.1	65.7
Acacia, *Robinia*	4.5	23.5	7.4	68.5
Blackberry, *Vaccinium*	4.3	127.4	27.6	78.3
White clover, *Trifolium*	4.1	332.5	44.1	86.7
White clover, *Trifolium*	4.0	182.0	9.8	94.6

a = accumulation of µg H_2O_2 • g^{-1} $hour^{-1}$; b = mostly honeydew flow

Honeydew honeys which have a higher pH (usually over 5), are less sensitive than the relatively acid floral honeys. Honeys of the same colour and with the same pH value have however been found to be sometimes very differently sensitive to light. It is assumed that certain substances exist in some honeys which increase their sensitivity to light (their chemical nature is not known). The effect of light may be so strong as to destroy the enzyme which produces peroxide, with no inhibine effect existing any more, even in the 500 g jar when exposed to sunlight for a longer time in the shop window: after keeping a jar with a honey mixture of *Brassica*, *Trifolium* and *Potentilla* in the sunlight for 48 hours, no trace of enzyme activity was recorded to exist anywhere in the honey mass. Investigation of different honey samples showed that honeys are 15 % less sensitive to the light of an incandescent lamp (3000 lux) than to the fluorescent light of the same intensity. As the honey packed in white glass jars is often exposed to fluorescent light in shop windows for several months, it is very likely that much of this honey has none of its praised natural qualities any more when the consumer buys it. There is also the effect of heat which inactivates the enzyme as well, as reported by WHITE.

All these findings show that the secretion – including glucose oxidase, of the hypopharingeal glands of bees plays a part of overriding importance in the antibacterial effect of honey.

The antibacterial effect conditioned by other factors than the enzyme activity and sugar content in honey is very small. It was found that in acetone extracts of floral and honeydew honeys the antibacterial effect was a small fraction only of the antibacterial effect resulting from peroxide accumulation (often less than 1/50). In conclusion we point out once again that the antibacterial effect of honey varies substantially depending on the honey type. As shown above, with honeys with high inhibine values very small amounts of honey are sufficient to provide for an antibacterial effect. Greater care should be taken with respect to the sensitivity of the natural honey to light and other factors which negatively affect its quality.

By our studies we wish to provide in the future for the control of certain important pathogen germs by means of honeys with high inhibine values.

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