BRMI
Bioregulatory Medicine Institute

Apitherapy

Products of the Hive

Honey bees are master chemists and chemical engineers. Their 40-million-year success in the animal kingdom is largely because of the chemistry and the application of their products: honey, pollen, royal jelly, propolis, beeswax, and venom. The bees themselves chemically synthesize three of these products, beeswax, venom, and royal jelly. The other three are derived from plants and are modified and engineered by the bees for their own use.

Honey

History of Usage

Honey was almost the only source of sugar available to people in ancient times, and was highly valued for its medicinal benefits. It was used to make mead, a fermented beverage, and was mixed with wine and other alcoholic drinks. In Egypt it was also employed as an embalming material. Aristotle (c.350 BC) wrote that “Spring honey is sweeter, whiter, and in every way better than the autumn honey” and “White honey does not come from thyme pure and simple; it is good as a salve for sore eyes and wounds”. Dioscorides (first century AD) wrote that “the best honey is from Attica, the best of that from Mount Hymettus, next best is from the Cyclad Islands and from Sicily. Spring honey is best, then Summer honey” and that “honey that is sharp, of a fragrant smell, is pale yellow and thixotropic is good for all rotten and hollow ulcers”. There is a similar awareness in present-day folk medicine: in Sardinia strawberry-tree honey, and in Dubai honey from the Jirdin valley of Yemen, are highly valued for their therapeutic properties. In India, lotus honey is said to be a panacea for eye diseases. In New Zealand it was the long-standing reputation of Manuka honey for its antiseptic properties that prompted the laboratory investigations that led to it being found that it had an unusual antibacterial component. In laboratory studies where multiple honeys have been tested against the same strain of bacteria, up to a hundred-fold difference in antibacterial potency has been found. Large variations in the content of antioxidants in honeys (which also are probably of importance in wound healing) have also been found. Aside from its long history of use as a sweetener and medicine, honey has evolved other commercial uses such as in cosmetics, meatpacking, and curing tobacco.

Current Production

Since humans first began keeping bees, their principal aim has been the harvest of honey. Thus, beekeeping methods have been adapted to accommodate colony behavior. Some success has been achieved in manipulation of colonies to capitalize on

certain behavioral traits, but honey bees must still be considered wild, not domesticated, creatures, and honey a raw agricultural commodity. Ninety percent of the world's beekeepers live in Europe, Russia, Asia, and Africa and produce 54 percent of the world's honey by averaging 9.1 kg (20 lb.) per colony. Ten percent of the world's beekeepers live in Australia and North and South America and produce 46 percent of the world honey crop by averaging 22.7 to 45.4 (50 to 100 lb.) per colony. Some stingless bees (not honey bees) are kept for honey production, but the number of these colonies is comparatively small. Worldwide honey production is currently in excess of 600,000 tons annually. This quantity is provided by an estimated 50 million colonies kept by about 6.5 million beekeepers. The current world population is around 6.3 billion people, and it is estimated that there are 500 times more honey bees than people.

Chemical Properties and Composition

Honey is elaborated from the nectar of numerous plant species but may also be produced from honeydew excretions of aphids and scale insects. Nectars vary considerably in quality and quantity, depending on the floral source. Similarly, honey varies; some honey is nearly colorless (like water), with a light, pleasing aroma, and some is as dark as crankcase oil, with a heavy-bodied aroma. Honey from most floral sources falls between these extremes. Bees convert nectar to honey by drying it down to a moisture content of 15 to 20 percent and by adding a salivary enzyme that changes sucrose (long-chain sugar) into glucose and fructose (two short-chain sugars). Hence, honey is composed of sugars, mainly fructose and glucose. Honey also contains trace amounts of minerals, enzymes, vitamins, and colloids. Honey is water soluble, may granulate between 10° and 18°C, and is acidic.

The high acidity of honey also plays an important role in the system that prevents bacterial growth. The pH of honeys may vary from approximately 3.2 to 4.5 (average pH= 3.9). The acids of honey account for less than 0.5 percent of the solids, but this level contributes not only to the flavor, but is in part responsible for the excellent stability of honey against microorganisms. Several acids have been found in honey, gluconic acid being the major one. It arises from dextrose through the action of an enzyme called glucose oxidase. Other acids in honey are formic, acetic, butyric, lactic, oxalic, succinic, tartaric, maleic, pyruvic, pyroglutamic, *a*-ketoglutaric, glycolic, citric, malic, 2- or 3-phosphoglyceric acid, *a*- or *B*-glycerophosphate, and glucose 6-phosphate.

The amount of nitrogen in honey is low, 0.04 percent on the average, though it may range to 0.1 percent. Recent work has shown that only 40 to 65 percent of the total nitrogen in honey is in protein, and some nitrogen resides in substances other than proteins, namely the amino acids. Of the 8 to 11 proteins found in various honeys, 4 are common to all, and appear to originate in the bee, rather than the nectar. Little is known of many proteins in honey, except that the enzymes fall into this class. The presence of proteins causes honey to have a lower surface tension than it would have otherwise, which produces a marked tendency to foam and form scum and encourages formation of fine air bubbles. Beekeepers familiar with buckwheat honey know how readily it tends to foam and produce surface scum, which is largely due to its relatively high protein content.

One of the characteristics that differentiates honey from all other sweetening agents is the presence of enzymes. These conceivably arise from the bee, pollen, nectar, or even yeasts or microorganisms in the honey. Those most prominent are added by the bee during the conversion of nectar to honey. Enzymes are complex protein materials that under mild conditions bring about chemical changes, which may be very difficult to accomplish in a chemical laboratory without their aid. The changes that enzymes bring about throughout nature are essential to life. Some of the most important honey enzymes are invertase, diastase, and glucose oxidase.

Invertase, also known as sucrase or saccharase splits sucrose into its constituent simple sugars, dextrose and levulose. Other more complex sugars have been found recently to form in small amounts during this action and in part explain the complexity of the minor sugars of honey. Although the work of invertase is completed when honey is ripened, the enzyme remains in the honey and retains its activity for some time. Even so, the sucrose content of honey never reaches zero. Since the enzyme also synthesizes sucrose, perhaps the final low value for the sucrose content of honey represents equilibrium between splitting and forming sucrose.

Diastase (amylase) digests starch to simpler compounds but no starch is found in nectar. What its function is in honey is not clear. Diastase appears to be present in varying amounts in nearly all honey and it can be measured. It has probably had the greatest attention in the past, because it has been used as a measure of honey quality in several European countries.

Glucose oxidase converts dextrose to a related material, a gluconolactone, which in turn forms gluconic acid, the principal acid in honey. Since this enzyme previously was shown to be in the pharyngeal gland of the honey bee, this is probably the source. Here, as with other enzymes, the amount varies in different honeys. In addition to gluconolactone, glucose oxidase forms hydrogen peroxide during its action on dextrose, which has been shown to be the basis of the heat-sensitive antibacterial activity of honey. Other enzymes are reported to be present in honey, including catalase and an acid phosphatase. All the honey enzymes can be destroyed or weakened by heat.

The low moisture content of honey is one of its most important characteristics as it influences keeping quality, rate of granulation and body. Honey is hygroscopic (absorbs moisture) and will remove moisture from the air if the relative humidity exceeds 60 percent. Care must be taken in the handling and storage of honey to be sure that this does not happen. However, hygroscopicity is one of the traits the makes honey desirable for baking; goods sweetened with honey will stay moist longer. The low moisture content of honey also forms an important part of the system that protects honey from attack by microorganisms. Honey's hyperosmotic nature due to the high concentration of solids and low moisture content prevents the growth of bacteria and yeasts as it draws water out of the organisms.

Medicinal Properties and Usage of Honey

Antimicrobial Effect in Wound Healing

There is rapidly increasing interest in the use of honey as a wound dressing because of its properties of rapid clearance of infection (including infection with antibiotic-resistant bacteria), rapid debridement of wounds, rapid suppression of inflammation, minimization of scarring and stimulation of angiogenesis and the growth of granulation tissue and epithelium. Early Egyptians were the first to use honey as a component (along with animal fats and vegetable fibers) in the topical treatment of wounds as evidenced from their writings in the Smith papyrus (1650 BC). In the time of Aristotle, it was recommended that honey collected in specific regions and seasons (and therefore presumably from different floral sources) be used for the treatment of particular ailments. Although it appears that the honey from certain plants has better antibacterial activity than that from others, little work has been done to measure these variations. Honeydew honey from the conifer forests of the mountainous regions of central Europe has been found to have particularly high antibacterial activity, likewise honey from manuka (*Leptospermum scoparium*) in New Zealand has been found to have special antibacterial properties. Studies on the effectiveness against wound-infecting species of bacteria show that manuka honey is more effective than other honeys for *Escherichia coli* and *Staphylococcus aureus* while other honey was superior for the other 5 tested species, including *Salmonella, Streptococcus*, and *Pseudomonas.* There was little difference between the two types of antibacterial activity in their effectiveness, although some bacteria were more sensitive to the action of one type of honey than the other.

Rose Cooper of the University of Wales Institute, Cardiff, and colleagues at the University of Waikato, New Zealand, have tested natural honey and an artificial honey solution of sugars against dozens of strains of antibiotic-resistant bacteria. They found that honey kills several wound-infecting bacteria, such as the notorious MRSA (methicillin-resistant *Staphylococus aureus*) and enterococcal bacteria resistant to the potent antibiotic vancomycin. The synthetic solutions failed.

ABSTRACT

J. Wound Care, April 1999;8(4):161-164.

"The Use of Honey as an Antiseptic in Managing Pseudomonas Infection," Cooper R, Molan P

In isolating Pseudomonas from 20 infected wounds and placing them in pure cultures with various concentrations of Manuka honey and pasture honey, the minimum inhibitory concentration of Manuka honey for the 20 isolates ranged from 5.5-8.7% (v/v). The minimum inhibitory concentration of pasture honey for the 20 isolates ranged from 5.8-9.0% (v/v). These data suggest that honeys with average antibacterial activity would be expected to prevent the growth of Psuedomonas on the surface of a wound, even if the honeys were diluted more than 10-fold by the exudates that are produced by the wound.

ABSTRACT

J R Soc Med, June 1999;92:283-285.

"Antibacterial Activity of Honey Against Strains of *Staphylococcus aureus* From Infected Wounds," Cooper RA, Molan PC, Harding KG
In evaluating 58 strains of coagulase-positive *Staphylococcus aureus* which were isolated from infected wounds, utilizing a pasture honey and a manuka honey, minimum inhibitory concentrations were between 2% and 3% (v/v) for the manuka honey and between 3% and 4% for the pasture honey. These honeys could still prevent the growth of *Staphylococcus aureus* if diluted by body fluids by an additional 7- to 14-fold beyond the point where their osmolarity ceased to be completely inhibitory. Pasture honey maintains its antibacterial action through the release of hydrogen peroxide. Manuka honey additionally has a phytochemical component, which may inhibit *Staphylococcus aureus* growth.

Honey sold especially for wound care is now standardized for its antibacterial activity. Although all honey has antibacterial activity due to its high osmolarity, it soon fails to suppress growth of bacteria on a wound as it becomes progressively diluted by exudate. Whereas a honey with no antibacterial effect beyond the osmolarity would cease to stop the growth of Staphylococcus aureus once diluted four-fold or more, honey sold especially for wound care would still be inhibitory if diluted more than sixty-fold.

Generally, all honey has an antibacterial activity not only because of its high osmolarity but also due to hydrogen peroxide formed in a slow-release manner by the presence of the enzyme glucose oxidase.

Recipe for Honey Wound Dressing
Some practical considerations are:

- Ensure that there is an even coverage of the wound surface with honey. Honey can be made fluid by stirring or warming. Cavities may be filled by pouring in fluidized honey, or more conveniently by using honey packed in squeeze-tubes. (Gamma-irradiated manuka honey in tubes is available commercially.)
- Spread the honey on the dressing pad rather than on the skin lesion. This is much easier to do and causes less discomfort for the patient.
- The amount of honey needed depends on the amount of fluid exuding from the wound. The benefits of honey on wound tissues will be reduced if honey becomes diluted. Typically, 20 ml of honey is used on a 10 cm X 10 cm dressing.
- Cover the wound with absorbent secondary dressings to prevent honey oozing out from the dressing. Change the dressings more frequently if the honey is being diluted with secretions. Otherwise change the dressing every day or two.

Antimicrobial Effect in Peptic and Duodenal Ulcers
Manuka honey is gathered in New Zealand from the manuka bush, Leptospermum scoparium, which grows uncultivated throughout the country. More recently, as a result of systematic screening of Australian honeys, a honey with the same properties is produced from Leptospermum polygalifolium, which grows uncultivated in a few parts of Australia.

Manuka honey is being used to treat dyspepsia and peptic and duodenal ulcers. Dr Peter Molan, Associate Professor of Biochemistry at the University of Waikato, New Zealand, has extensively researched the antibacterial properties of manuka honey. During a decade of research at the university's Honey Research Unit, Dr. Molan has isolated the active properties in manuka honey that inhibit the growth of helicobacter pylori (the bacteria that causes peptic and duodenal ulcers).

Not all manuka honey has this antibacterial activity, hence, Manuka honey is now laboratory tested and rated for its antibacterial effects. 'Unique manuka factor' (UMF) is the current rating used to indicate the levels of antibacterial properties. The UMF numbers come from a standard laboratory test of antibacterial activity, with honey being compared with a standard antiseptic (phenol) for potency. For example, a honey with a UMF rating of 4 would be equivalent to the antiseptic potency of 4% solution of phenol, a carbolic disinfectant; a honey with a rating of 10 would have potency equivalent to a 10% solution of phenol. To alleviate any concern over the possible risk of introducing infection using an unprocessed natural product on wounds, honey can be sterilized by gamma irradiation without loss of any of its antibacterial activity.

The catalase enzyme present in body tissue and serum does not affect unique manuka factor. This enzyme will break down, to some degree, the hydrogen peroxide which is the major antibacterial factor found in other types of honey. If a honey without UMF were used to treat an infection, the potency of the honey's antibacterial activity would most likely be reduced because of the action of catalase. The enzyme that produces hydrogen peroxide in honey is destroyed when honey is exposed to heat and light. But UMF is stable, so there is no concern about manuka honey losing its activity in storage. Also the enzyme that produces hydrogen peroxide in honey becomes active only when honey is diluted. UMF is active in full strength honey, which will provide a more potent antibacterial action diffusing into the depth of infected tissues. A UMF rating of 10 - 15+ proves effective against H. Pylori and many other pathogenic bacteria.

Pollen

Chemical Properties and Composition

In its original state, pollen is a fine powder composed of thousands of microscopic particles that are discharged from the anther of a flower, and is the male element of the plant that fertilizes like plants. There are two kinds of pollen: anemophile ("friend of the wind"), and entomophile ("friend of the insect"). The air disperses the former, which causes allergic reactions like hay fever. The latter, which is the subject of this information, is gathered by the honey bee, whose travels from flower to flower make possible the reproduction of more than 80% of the world's grains, fruits, vegetables and legumes.

The individual pollen grain is encased in two protective coatings. The external layer is composed of sporopollen and cellulose and is known to be acid-resistant and may

withstand temperatures more than 300 degrees C. Beneath this is the inner layer, which preserves oil and starch.

The honey bee collects only the purest of pollens. It avoids all toxic plants, including those contaminated with pesticides. The honey bee collects pollen and mixes it with its own digestive enzymes. One pollen granule contains from one 100,000 to 5,000,000 pollen spores, each capable of reproducing its entire species.

One of the most interesting facts about bee pollen is that it cannot be duplicated in the laboratory. Thousands of chemical analyses have been done with the latest in diagnostic equipment and there are still (at least 2%) unidentified elements present in pollen. These unidentifiable elements may account for why bee pollen works against so many different health conditions.

Medicinal Properties and Usage of Bee Pollen

Nutritional tests have been performed that demonstrate pollen to be a complete food. These were done by letting several successive generations of mice be born and live without the least sign of distress while nourishing them exclusively on pollen. It has also been noted that because bee pollen stimulates the production of hemoglobin it has a favorable action on anemia.

Bee pollen has been effectively used since ancient times to rid allergy sufferers of their affliction. This technique is called “desensitization”, which consists of administering small amounts of an allergen to stimulate the body’s own immune system to produce antibodies that will eliminate the allergic reaction. Though local pollen may be best for desensitization, any honey bee pollen will work.

Human consumption of bee pollen is praised in numerous religious texts, i.e., the Bible, and in ancient Chinese and Egyptian writings. Research studies document the therapeutic efficacy and safety of bee pollen. Clinical tests show that orally-ingested bee pollen particles are rapidly and easily absorbed. Pollen passes directly from the stomach into the blood stream and within two hours after ingestion, bee pollen is found in the blood, in cerebral spinal fluids, and in the urine.

Pollen has also been successfully used for treatment of some cases of benign prostatitis.

ABSTRACT:

Clinical evaluation of Cernilton in benign prostatic hypertrophy

Hayashi J, Mitsui H, Yamakawa G, Suga A, Kai A, Shimabukuro T, Yanagi K, Fujisawa S, Takihara H, Kaneda Y, et al

Twenty patients with benign prostatic hypertrophy were treated with Cernilton (honey bee pollen extract), 6 tablets a day for an average of 13.2 weeks. Subjective effectiveness was observed in the improvement of sense of residual urine (92%), retardation (86%), night frequency (85%), strain on urination (56%), protraction (53%) and forceless urinary stream (53%). The overall subjective effectiveness was 80% of

patients, and the overall objective effectiveness was 54% of patients. Night frequency, residual urine volume and tidal urine volume were improved significantly. The overall effectiveness was 80%. No side effects were observed.

Each ounce of honey bee pollen contains just 28 calories; 7 grams are carbohydrate, plus 15% Lecithin, and 25% protein.

From the following composition list it is easy to understand why pollen is called nature's most complete food, "A food fit for the Gods'. During the collection process the bees produce digestive enzymes, which become a component of the bee pollen. Bee pollen affects all body systems with special benefit to nervous systems, reproductive and immune systems.

"Bee" warned however, that some individuals are sensitive to pollen and caution should be taken when beginning to consume it.

Chemical Analysis of Honey Bee Pollen

Vitamins:
Provitamin A (carotenoids) 5-9 mg %
Vitamin B1 (thiamine) 9.2 micrograms %
Vitamin B2 (riboflavin)
Vitamin B3 (niacin)
Vitamin B5 (panothenic acid)
Vitamin B6 (pyridoxine) 5 micrograms %
Vitamin B12 (cyanocobalamin)
Vitamin C (ascorbic acid)
Vitamin D; Vitamin E
Biotin
Vitamin K; Choline; Inositol
Folic Acid, 5 micrograms %
Pantothenic acid 20-50 micrograms/gram
Rutin 16 milligrams %, Rutin in beehive pollen 13%
Vitamin PP (nicotinicamide)

Minerals:
Calcium 1 - 15% of ash
Phosphorus 1-20% of ash
Iron, 1-12% of ash, 0.01-1.3% of fresh pollen
Copper 05-08% of ash
Potassium, 20-45% of ash
Magnesium, 1-12% of ash
Manganese, 1.4% of ash, 0.75 mg %
Silica, 2-10% of ash
Sulphur, 1% of ash
Sodium
Titanium
Zinc
Iodine
Boron
Molybdenum

Fatty Acids (Conifer Pollen)
Total list identified are:
Caproic (C-6)
Caprylic (C-8)
Capric (C-10)
Lauric (C-12)
Myristic (C-14)
Palmitic (C-16)
Stearic (C-18)
Oleic (C-18) one double bond
Linoleic (C-18) two double bonds
Linolenic (C-18) three double bonds
Eicosanoic (C-20) one double bond
Arachidic (C-20)
Stearic (C-22)
Brucic (C-22) one double bond

Dry Pollen Contains:
0.76-0.89 % fatty acid. Major are:
Oleic, Palmitic, Linoleic,
Pinus dry pollen contains:
125-1.33% fatty acid based on
dry weight of pollen, major are:
Linolenic, Oleic, Stearic

Fats & Oils: - 5%
Fatty acid (may be 5.8%)
Hexadecanol may be 0. 14% of pollen by weight.
Alpha-amino butyric acid is present in pollen fat.
Unsaponifiable fraction of pollen may be
2.6% by weight.
Water: 3-20% of fresh pollen

Proteins, Globulins, Peptones, and Amino Acids:
7-35%. Average 20%: 40-50%
may be free amino acids: 10-13% consists
of amino acids in dry pollen.
35 grams of pollen per day can satisfy the protein requirements of man. 25 grams of pollen per day can sustain man because it contains 6.35 grams as indicated by Rose.
Plus other amino acids.

Carbohydrates:
Gums - Pentosans - Cellulose Sporonine
(7-57% of pollen of various species:
29% in bee collected.)
Starch (0-22% of pollen)
Total sugars (30-40%)
Sucrose, levulose, fructose, glucose

Enzymes & Co-enzymes:
Bee pollen contains all known enzymes & co-enzymes A few are listed below:
Disstase
Phosphatase
Amylase
Catalase
Saccharase
Diaphorase
Pectase
Cozymase
Cytochrome systems
Lactic dehydrogenase
Succinic dehydrogenase
Note: The cozymase in mixed fresh pollen runs about 0.5-1 .0 milligram per gram comparable to the amounts in yeast.

Pigments:
Xarmmepayll (20-150 micrograms per gram)
Carotenes (50-150% micrograms per gram)
Alpha & Beta Carotene

Miscellaneous:
Waxes, Resins, Steroids, Growth Factors, Growth Isorhanetin, Vernine, Guanine, Xanthine, Hypoxanthine, Nuclein, Amines, Lecithin, Glycoside of Isorhanstin, Glycosides of Quercetin, Selenium, Nucleic acids flavonoids, phenolic acids, tarpenes and many other yet undefined nutrients.

Pollen contains the same number of amino acids, but vary greatly in quantity of each:
Tryptophan 1.6% - Leucine 5.6% Lysine 5.7% - Isoleucine 4.7% Methionine 1.7% - Cystine 0.6% Threonine 4.6% - Arginine 4.7% Phenylalanine 3.5% - Histidine 1.5% Valine 6.0% - Glutamic acid 2.1% Tyrosine - Glycine - Serine - Proline - Alanine - Aspartic acid Hydroxyproline - Butyric Acid.

Other micronutrients are:
Nucleosides; Guanine; Hexodecanol; Auxins
Xanthine; Alpha-Amino-Butyric Acid
Brassins; Hypoxalthine; Monoglycerides
Gibberellins; Crocetin; Diglycerides
Kinins; Zeaxanthin; Triglycerides; Vernine
Lycopene; Peutosaus

Royal Jelly

Royal jelly is the substance that turns an ordinary bee into a queen bee. It is made of pollen that is chewed up and mixed with a chemical secreted from a gland in the nursing bee's heads. This "milk" or "pollen mush" is fed to all the larvae for the first two days of their lives. The larva chosen to become a queen continues to eat only royal jelly. The queen grows one and a half times larger than workers, and can lay up to two thousand eggs a day. The queen bee lives forty times longer than the bees on a regular diet. There is no difference between a queen bee and a worker bee in the larval stage. The only factor that is different between them is that a developing queen bee continues to eat only royal jelly.

Chemical Properties and Composition

Royal jelly is an emulsion of proteins, sugars and lipids in a water base, and is synthesized by the bee from pollen. 82-90% of the protein content is made up of a group of proteins found only in royal jelly and worker jelly, known as the Major Royal Jelly Proteins (MRJPs), which has five main members. These are rich in the essential amino acids (i.e.. those amino acids which cannot be biosynthesized), and so play an important role in bee nutrition. The MRJPs do not show any enzyme activity or other special properties, and so they do not seem to have any role other than nutrition. Most of the components of royal jelly seem to be designed to provide a balance of nutrients for the larvae. However, the lipids present are unusual, in that they are unlike the lipids of typical insect fats, which consist of 14-20 carbon fatty acids. Royal jelly lipids are composed mainly of 8-10 carbon acids, hydroxy acids and diacids, which may be saturated, unsaturated, linear or branched. They include hexanoic acid, octanoic acid, (E)-oct-2-enoic acid, 8-hydroxyoctanoic acid, 3- and 10-hydroxydecanoic acid, and 3, 10-dihydroxyoctanoic acid. 10-hydroxydecanoic acid levels rise dramatically in summer. Royal jelly also contains 7-9 sterols, 4 phospholipids, 5 glycolipids, and a variety of 16-33 carbon hydrocarbons. The unusual lipids of royal jelly make it highly acidic, and give it good antimicrobial properties. This seems to be the main role of the lipids. However, these properties disappear above pH 6, so while royal jelly may be used as an effective skin-care product, its antimicrobial properties are negligible within the body, where the pH is maintained at about 7.4 by buffering systems. Its unique balance of nutrients and high nutrient levels accounts for it becoming a highly-touted specialized health food.

Chemical analysis of royal jelly confirms its richness in elements such as B vitamins (B1, B2, B6, B12, PP, H, and folic acid), minerals (phosphorous, copper, iron, sulphur, and selenium), unsaturated fatty acids, amino acids and hormonal substances. The composition of 100 g. contains: proteins 48.2 g., fats 10.4 g., sugars 38.8 g., B vitamins, vitamin C and A, minerals, antitoxic and antibiotic elements.

Medicinal Properties and Usage of Royal Jelly

Scientists decided to try feeding the queen bee's diet to other animals with surprising results. The life span of pigs and roosters showed as much as a 30% increase. Fruit flies fed royal jelly increased in size and in rate of production. Chickens given royal jelly laid twice as many eggs, and older chickens began to lay again. In France, there have been reports of women fed royal jelly during menopause, showing complete remission

of their symptoms. Some were even able to become mothers again. France also claimed that their studies showed royal jelly to have rejuvenating and sexually stimulating effects on both men and women. Canada has approved royal jelly as a natural dietary supplement for its athletes. Royal jelly is not a drug, but a nutritious, quickly assimilated food. In Germany, Drs. Chochi, Prosperi, Quadri and Malossi (in separate studies) used royal jelly as an aid to undernourished and premature babies. The infants fed royal jelly significantly and rapidly increased in weight and health. Another researcher reported that neuro-psychic patients given royal jelly regained normal weight, had a more stable nervous system, and exhibited more stamina for physical and mental work.

Royal jelly has proven to be a potent bactericide and is clinically shown to speed up healing of wounds and to reduce the amount of scarring.

ABSTRACT
J Biol Chem. 1990 Jul 5;265(19):11333-7.
A potent antibacterial protein in royal jelly. Purification and determination of the primary structure of royalisin. Fujiwara S, Imai J, Fujiwara M, Yaeshima T, Kawashima T, Kobayashi K.
Biochemical Research Laboratory, Morinaga Milk Industry Company Limited, Kanagawa, Japan.
A new potent antibacterial protein, for which we propose the name royalisin, was found in royal jelly of the honeybee Apis mellifera L. and purified to homogeneity for the first time by acid extraction, gel filtration, and reverse-phase high pressure liquid chromatography. The primary structure of royalisin was determined to consist of 51 residues, with three intramolecular disulfide linkages, having a calculated molecular mass of 5523 Da. Royalisin is an amphipathic protein, with the C-terminal half of the molecule being rich in charged amino acids; and it showed extensive sequence homology to two other antibacterial proteins, sapecin from embryonic Sarcophaga peregrina cells and phormicins from Phormia terranovae larvae. Royalisin was found to have potent antibacterial activity against Gram-positive bacteria at low concentrations, but not against Gram-negative bacteria. Royalisin may be involved in a defense system active against bacterial invasion of the honeybee.

ABSTRACT
Jpn J Pharmacol. 1990 Jul;53(3):331-7.
Augmentation of wound healing by royal jelly (RJ) in streptozotocin-diabetic rats.
Fujii A, Kobayashi S, Kuboyama N, Furukawa Y, Kaneko Y, Ishihama S, Yamamoto H, Tamura T.
Department of Pharmacology, Nihon University School of Dentistry, Matsudo, Japan.
Chronically diabetic rats prepared by a single intravenous injection of streptozotocin were used to study whether royal jelly (RJ) possesses a hypoglycemic reaction and whether it can augment wound healing. Oral RJ administration of 10, 100 and 1000 mg/kg/day did not show any insulin-like activity (the hypoglycemic reaction). RJ, however, showed some anti-inflammatory activity by decreasing exudation and collagen formation

in granulation tissue formation in the cotton pellet method. RJ also shortened the healing period of desquamated skin lesions. Thus, RJ possesses an anti-inflammatory action and is able to augment wound healing, but does not have an insulin-like action in streptozotocin-diabetic rats.

The beneficial effects of royal jelly seem not to depend entirely upon its vitamin content, but upon some type of enzymatic or catalytic action of an as yet unknown factor; or perhaps, the known factors working in combination with a co-enzyme through a process that has not yet been identified. Since the action of royal jelly seems to be systemic rather that one which affects a specific biological function, it has been recommended for a great variety of purposes: to retard the aging process, for menopause, correction of malnutrition, arthritis, vascular diseases, peptic ulcers, liver ailments, nervous instability, skin problems, improvement of sexual function, and general health and well being.

As with propolis, royal jelly also has anti-tumor properties.

ABSTRACT
Nippon Yakurigaku Zasshi. 1987 Feb;89(2):73-80.
[Antitumor effects of royal jelly (RJ)][Article in Japanese]
Tamura T, Fujii A, Kuboyama N.
Anti-tumor effects of Royal Jelly (RJ) were investigated employing the transplantable tumors of mouse advance leukemia L1210 and P388 strains and Ehrlich, Sarcoma-180 ascites and solid tumor strains. RJ was administered orally in a prophylactic-therapeutic (30 days before and 30 days after the transplantations of tumor cells) or a therapeutic (30 days after the transplantations of tumor cells) manner. Tumor cells were transplanted i.p. (ascites tumor) or s.c. (solid tumor). The daily dose of RJ was 0 (control), 10, 100, or 1000 mg/kg. In the case of the therapeutic experiments employing advance leukemia L1210 and P388 strains, which gave quite a short survival period of 8 approximately 9 days, RJ did not show any anti-tumor effect. In the case of the therapeutic RJ application employing the Sarcoma-180 ascites tumor, which gave a moderate survival period of 16 days, the increased life span was 9.3 approximately 19.3%; and with the Ehrlich ascites tumor (survival period of 22.1 days), the increased life span was 20.4% (RJ 10 mg/kg. day) and 17.6% (RJ 1,000 mg/kg. day), but no anti-tumor effect was observed at the dose of 100 mg/kg. day. In the case of the therapeutic experiment employing Ehrlich solid tumor, tumor growth inhibition was 25.3 approximately 54.8%, whereas the use of the prophylactic-therapeutic regimen gave a tumor growth inhibition of 38.3 approximately 45.7%. In the case of the therapeutic RJ application employing Sarcoma-180 solid tumor, tumor growth inhibition was 45.1 approximately 59.7%, whereas the prophylactic-therapeutic regimen gave a tumor growth inhibition of 49.1 approximately 56.1%.

Available as:
Royal jelly may be purchased as a freeze-dried product. For better taste the freeze-dried royal jelly may be mixed with a local uncooked, unfiltered honey.

“Bee” warned however, that some individuals are sensitive to royal jelly and caution should be taken when beginning to consume it.

Propolis

Propolis is a resinous yellow-brown to dark brown substance collected by worker honey bees from the growing parts of trees and shrubs (e.g., leaf buds, trunk wounds). The bees pack the propolis on their hind legs, and carry it back to their colony, where it is combined with beeswax and used by worker "hive" bees as a sealant and sterilant in the colony nest. These workers take the resinous material and add salivary secretions and wax flakes to it, then use the new product for numerous protective purposes as bee propolis. The bees use it to coat the inside of the hive, including the passageway and the brood chambers.

Propolis protects the hive in two ways: First, it reinforces the hive itself; second, it protects the hive from bacterial and viral infection. And it is these latter properties which man has found so helpful through the centuries. Propolis has also been shown to kill *Bacillus larvae,* the most important bacterial disease of bees (Mlagan and Sulimanovic, 1982).

Propolis changes consistency with temperature. At temperatures below 15^0C it is hard and brittle, but becomes more pliable and sticky at higher temperatures (25-45^0C). Propolis generally melts at 60-70^0C, although some samples have been found to have a melting point as high as 100^0C (Krell, 1998).

History of Propolis Usage

Bees have used propolis for millions of years, and humans have used it for thousands. Both species find it immensely useful and beneficial. Much of the bees' success in surviving through the ages may be credited to propolis. As humans, we may yet discover we've only just scratched the surface of the benefits of this resinous wonder.

The Greek physician, Hippocrates, prescribed the use of propolis to help heal internal and external sores and ulcers. Ancient Egyptians depicted propolis-making bees on vases and other ornaments, and used the resinous substance to alleviate many ailments. Pliny, the Roman scholar, wrote much on the use of resins such as propolis in his massive book, *Natural History*. He touts the abilities of propolis to reduce swelling, soothe pain, and heal sores, to name a few.

In *The History of Plants* written by John Gerard in 1597, propolis was noted for its ability to provide swift and effective healing for many conditions. During this era, propolis was used in many different healing ointments. Propolis has been used since early times, for various purposes, and especially as a medicine because of its antimicrobial properties (Crane, 1997).

Ancient Greek texts refer to the substance as a "cure for bruises and suppurating sores", and in Rome physicians in making poultices used propolis. The Hebrew word for propolis is “tzori”, and the therapeutic properties of tzori are mentioned throughout the Old Testament. Records from 12th century Europe describe medical preparations using propolis for the treatment of mouth and throat infections, and dental caries (Krell, 1996).

Propolis is collected by commercial beekeepers, either by scraping the substance from wooden hive parts, or by using specially constructed collection mats. The raw product undergoes secondary processing to remove beeswax and other impurities before being used in a variety of natural health care products (e.g., lozenges, tinctures, ointments, drinks).

One of the non-medicinal uses of propolis is as a varnish, and it has been suggested that the special properties of Stradivarius violins may be partly due to the type of propolis used, although the claim cannot be substantiated.

Chemical Properties and Composition of Propolis

Propolis is made up of phenolic compounds, beeswax, lipids and wax, flavonoids, terpenes (e.g. B-eudesmol), bio-elements (e.g. manganese, zinc, and copper), and other substances. One of these “other substances” 3-›4-hydroxy-3,5-bis(3-methyl-2-butenyl) phenyl-2-propenoic acid, or "Artepillin C", has been found to show antiseptic activity, hair growth activity, and anti-tumor activity.

At least 180 different compounds have been identified so far in propolis. A list of the major chemicals occurring in propolis is given in the following table (Krell, 1998):

Class of Compound	Group of Components	Amount
Resins	**Flavonoids, phenolic acids and esters**	**45-55%**
Waxes and Fatty |Acids	**Beeswax and Plant Origin**	**23-35%**
Essential Oils	**Volatiles**	**10%**
Pollen	**Proteins (16 free amino acids>1%) arginine and proline together 46% of total**	**5%**
Other Organics and Minerals	**14 trace minerals, iron and zinc most common; ketones, lactones, quinones, steroids, benzoic acid, vitamins and sugars**	**5%**

The most important pharmacologically-active constituents in propolis are the flavones, flavonols, and flavanones (collectively called flavonoids), and various phenolics and aromatics. Flavonoids play a major role in plant pigmentation. However, the flavonoids present in propolis are different in composition to those normally found in plants, since

propolis flavonoids are not glycosides (i.e., they do not have sugar molecules attached to their chemical structure). Many flavonoids found in plants are glycosides.

Flavonoids are thought to account for much of the biological activity in propolis (Grange and Davey, 1990), although other phenolic compounds are also involved. At least 38 flavonoids have been found in propolis, including galangin, kaempferol, quercetin, pinocembrin, pinostrobin and pinobanksin (Schmidt and Buchmann, 1992). Some of the other phenolics include cinnamic alcohol, cinnamic acid, vanillin, benzyl alcohol, benzoic acid, and caffeic and ferulic acid.

The chemical composition of propolis is highly variable because of the broad range of plants visited by honey bees when collecting the substance. Crane (1990) identifies at least 67 species from which honey bees have been reported to collect propolis material. Important sources include poplars, alders and birches, chestnut, ash, various Prunus and willows. Variations in the beeswax content of raw propolis also affect the chemical composition.

Studies indicate that the plant resins collected by bees are at least partially altered by bees prior to use in the hive. Propolis has little direct nutritive value, apart from the presence of small amounts of proteins, amino acids, minerals and sugars. Vitamins include small amounts of A, B_1, B_2, B_6, C and E (Ghisalberti, 1979). Dihydroflavonoids, like those found in propolis, have been shown to aid the human body in absorbing Vitamin C (Bors, et al, 1995).

Medicinal Properties and Usage of Propolis

Propolis is truly a fascinating compound that has a wide range of therapeutic applications. The following are specific therapeutic effects:

Antimicrobial Effects

Because of its strong antimicrobial activity, propolis is often known as a “natural antibiotic”. Many studies have shown an inhibitory effect on a variety of micro-organisms.

The antimicrobial effects are summarized in the following table (from Krell, 1996 and others):

Organism	**Comment**	**Reference**
Bacteria		
Bacillus larvae	destroyed	Mlagan and Sulimanovic 1982
Helicobacter pyiori	inhibited	Itoh et al 1994
MRSA	strong inhibition	Grange and Davey, 1990

Mycobacterium tuberculosis	Tb	Karimova 1975 Grange and Davey, 1990
Staphylococcus sp.	inhibited	Chernyak, 1973
Staphylococcus aureus	synergistic effect	Kedzia and Holderna. 1986
Streptococcus sp.	inhibited	Rojas and Cuetara, 1990
Streptomyces	inhibited	Simuth et al, 1986
S. sobrinus, mutans, cricetus	dental caries	Ikeno et al, 1991
Escherichia coli	inhibited	Simuth et al, 1986
Salmonella	potential treatment	Okonenko, 1989 and others
Giardia lambia	positive effect	Olarin et al, 1989 and others
Bacteroides nodosus	reduced foot rot	Munoz, 1989
Klebsiella pneumoniae	positive effect	Dimov et al, 1991
Fungi		
Candida albicans	synergistic effect	Holderna and Kedzia, 1987 and others
Botrytis cinerea	*in vitro* fungicidal	La Torre et al, 1990
Ascosphaera apis	inhibited	Ross, 1990
Viruses		
Herpes	inhibited *in vitro*	Sosnowski,1984
Potato virus	effective	Fahmy and Omar, 1989
Influenza (in mice)	reduced mortality	Serkedjieva, 1992 and others
Newcastle Disease	affected virus reproduction	Maksimova-Todorova et al 1985

Active components of propolis showing an antibacterial effect include pinocembrin, galangin, caffeic acid and ferulic acid. Antifungal components include: pinocembrin, pinobanksin, caffeic acid, benzyl ester, sakuranetin and pterostilbene. Anti-viral components include caffeic acid, lutseolin and quercetin (Schmidt and Buchmann, 1992). Propolis has been found to inhibit the synthesis of protein by bacteria, which may account for at least some of its antimicrobial effects (Simuth, et al, 1988).

Keeping in mind that the bees mix saliva or “bee lymph derived secretions” with plant resins in making propolis, the following abstract defines honey bee lymph as antimicrobial.

ABSTRACT
J. Biol. Chem., Vol. 268, Issue 10, 7044-7054, Apr, 1993

Functional and chemical characterization of Hymenoptaecin, an antibacterial polypeptide that is infection-inducible in the honeybee (Apis mellifera)
P Casteels, C Ampe, F Jacobs and P Tempst
Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, New York 10021.

As part of our ongoing search for novel antimicrobial agents and their use in singular or combined drug therapy, we have isolated a series of polypeptides from the lymph fluid of honeybees. These polypeptides are synthesized *de novo*, following experimental infection of the insect with live Escherichia coli cells, and confer a broad-spectrum antibacterial defense to the host. We have dissected this humoral "immune" system into its constituent components. In addition to the previously characterized apidaecins and abaecin, we also isolated a member of the defense family of peptide antibiotics and, now, a novel 93-amino acid long, cationic polypeptide, termed hymenoptaecin. Detailed analysis established the complete chemical structure, including a 2-pyrrolidone-5-carboxylic acid at the N terminus, and indicated major differences with all known antibacterial polypeptides. Under physiological conditions, it inhibits viability of Gram-negative and Gram-positive bacteria, including several human pathogens. Lethal effects against E. coli are secondary to sequential permeabilization of outer and inner membrane. In combination, the six-constituent "peptide antibiotics" of bee lymph provide wide-spectrum antibacterial protection in vitro by virtue of complementarity rather than synergism.

Anticancer Effects
Ethanol extracts of propolis have been found to transform human hepatic and uterine carcinoma cells *in vitro,* and to inhibit their growth. Substances isolated in propolis, which produce this cytotoxic effect, are quercetin, caffeic acid, and clerodane diterpendoid. Clerodane diterpendoid shows a selective toxicity to tumor cells.

ABSTRACT:
Antitumor activity of propolis extracted as micro-capsulated micelles (Meeting abstract)
Proc Annu Meet Am Assoc Cancer Res; 35:A2449 1994.
Matsuno T; Dept. of Virus Res. and Vaccine Control, Natl. Inst. of Health, Musashinurayama, Tokyo 208, Japan
The honeybee hive product, propolis, is a folk medicine employed for treating various ailments. It is alleged to exhibit a broad spectrum of activities including antibiotic, anti-inflammation, antioxidant and tumor growth arrest. We have isolated a new clerodane diterpenoid from Brazilian propolis, which inhibited DNA polymerase alpha activity and damaged various tumor cells. An excellent method of extracting propolis as micelles with fatty acid esters has been devised to facilitate the absorption. Effect of the

ingestion of this extract was investigated in cancer patients. The extract (30 to approx 40 ml/day) was harmless, and many patients felt they were physically in better condition. Immunological analyses of the peripheral lymphocyte subsets were carried out by cytofluorography. A promotive effect was frequently observed on the number of lymphoid cells with natural killer subsets (especially CD57- CD16+ and CD57+ CD16+) and CD3+ HLA-DR-. The extract considerably remitted various adverse effects of chemotherapy and radiation such as depletion of WBC and occurrence of inflammation of oral mucosa, nausea or falling-out of hair. Furthermore, it improved the survival and quality of life for many patients.

Propolis was also found to have a cytotoxic and cytostatic effect *in vitro* against hamster ovary cancer cells and sarcoma-type tumors in mice (Ross, 1990). The substance has also displayed cytotoxicity on cultures of human and animal tumor cells, including breast carcinoma, melanoma, colon, and renal carcinoma cell lines. (Grunberger et al, 1988). The component producing these effects was identified as caffeic acid phenethyl ester.

A substance called Artepillin C has also been isolated from propolis, and has been shown to have a cytotoxic effect on human gastric carcinoma cells, human lung cancer cells and mouse colon carcinoma cells *in vitro*.

ABSTRACT:

Apoptosis and Suppression of Tumor Growth by Artepillin C Extracted From Brazilian Propolis

Tetsuo Kimoto, M.D., Ph.D.,a Shigeyuki Arai, D.V.M., Ph.D.,a Michihiro Kohguchi, B.S., a Miho Aga, B.Pharm.,a Yutaka Nomura, B.S., a Mark J. Micallef, Ph.D.,a Masashi Kurimoto, M.S.,a and Keiichiro Mito, Ph.D.b

Fujisaki Institute, Hayashibara Biochemical Laboratories, Okayama, Japan; and Kawasaki College of Allied Health Professions, Kurashiki, Japan.

Artepillin C was extracted from Brazilian propolis. Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) has a molecular weight of 300.40 and possesses antibacterial activity. When artepillin C was applied to human and murine malignant tumor cells *in vitro* and *in vivo*, artepillin C exhibited a cytotoxic effect and the growth of tumor cells was clearly inhibited. The artepillin C was found to cause significant damage to solid tumor and leukemic cells by the MTT assay, DNA synthesis assay, and morphological observation *in vitro*. When xenografts of human tumor cells were transplanted into nude mice, the cytotoxic effects of artepillin C were most noticeable in carcinoma and malignant melanoma. Apoptosis, abortive mitosis, and massive necrosis combined were identified by histological observation after intratumor injection of 500 μg of artepillin C three times a week. In addition to suppression of tumor growth, there was an increase in the ratio of CD4/CD8 T cells, and in the total number of helper T cells. These findings indicate that artepillin C activates the immune system, and possesses direct antitumor activity.

ABSTRACT:

Inhibitory effects of propolis granular A P C on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice.

Sugimoto Y, Iba Y, Kayasuga R, Kirino Y, Nishiga M, Alejandra Hossen M, Okihara K, Sugimoto H, Yamada H, Kamei C.Department of Pharmacology, Faculty of Pharmaceutical Sciences, Okayama University, 1-1-1 Tsushima-naka, Okayama 700-8530, Japan.

We examined the effect of propolis granular A. P. C on lung tumorigenesis in female A/J mice. Lung tumors were induced by the tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) administered in drinking water for 7 weeks in mice maintained on an AIN-76A semi-synthetic diet. Propolis granular A. P. C (100 mg/kg body wt.) was administered orally daily for 6 days/week from 1 week before NNK administration and throughout the experiment. Sixteen weeks after the NNK treatment, the mice were killed and the number of surface lung tumors was measured. The number of lung tumors in mice treated with NNK alone for 7 weeks (9.4 mg/mouse) was significantly more than in that observed in control mice. Propolis granular A. P. C significantly decreased the number of lung tumors induced by NNK. These results indicate that propolis granular A. P. C is effective in suppressing NNK-induced lung tumorigenesis in mice.

Caffeic acid esters present in propolis have been shown to inhibit chemically induced tumor production in mice, as well as having a selective toxic effect on cells affected by genes, which promote the development of cancerous cells.

ABSTRACT:

Inhibitory effect of caffeic acid esters on azoxymethane-induced biochemical changes and aberrant crypt foci formation in rat colon

CV Rao, D Desai, B Simi, N Kulkarni, S Amin and BS Reddy

Division of Nutritional Carcinogenesis, American Health Foundation, Valhalla, New York 10595.

Previous work from this laboratory established that caffeic acid esters, present in the propolis of honey bee hives, are potent inhibitors of human colon tumor cell growth, suggesting that these compounds may possess antitumor activity against colon carcinogenesis. The present study was designed to investigate (a) the inhibitory effects of methyl caffeate (MC) and phenylethyl caffeate (PEC) on azoxymethane (AOM)-induced ornithine decarboxylase (ODC), tyrosine protein kinase (TPK), and arachidonic acid metabolism in liver and colonic mucosa of male F344 rats, (b) the effects of caffeic acid, MC, PEC, phenylethyl-3-methylcaffeate (PEMC), and phenylethyl dimethylcaffeate (PEDMC) on in vitro arachidonic acid metabolism in liver and colonic mucosa, and (c) the effects of PEC, PEMC, and PEDMC on AOM-induced aberrant crypt foci (ACF) formation in the colon of F344 rats. At 5 weeks of age, groups of animals were fed diets containing 600 ppm MC or PEC (biochemical study) or 500 ppm PEC, PEMC, or PEDMC (ACF study). Two weeks later, all animals except the vehicle-treated groups

were given s.c. injections of AOM, once weekly for 2 weeks. The animals intended for the biochemical study were sacrificed 5 days later and colonic mucosa and liver were analyzed for ODC, TPK, lipoxygenase, and cyclooxygenase metabolites. The animals intended for the ACF study were sacrificed 9 weeks later and analyzed for ACF in the colon. The results indicate that the PEC diet significantly inhibited AOM-induced ODC ($P < 0.05$) and TPK ($P < 0.001$) activities in liver and colon. The PEC diet significantly ($P < 0.001$) suppressed the AOM-induced lipoxygenase metabolites 8(S)- and 12(S)-hydroxyeicosatetraenoic acid (HETE). The animals fed the MC diet exhibited a moderate inhibitory effect on ODC and 5(S)-, 8(S)-, 12(S)-, and 15(S)-HETEs and a significant ($P < 0.001$) effect on colonic TPK activity. However, the MC and PEC diets showed no significant inhibitory effects on cyclooxygenase metabolism. In an in vitro study, caffeic acid and MC showed inhibitory effects on HETE formation only at a 100 microM concentration, whereas PEC, PEMC, and PEDMC suppressed in vitro HETE formation in a dose-dependent manner. AOM-induced colonic ACF were significantly inhibited in the animals fed PEC (55%), PEMC (82%), or PEDMC (81%). The results of the present study indicate that PEC, PEMC, and PEDMC, present in the propolis, inhibit AOM-induced colonic preneoplastic lesions, ODC, TPK, and lipoxygenase activity, which are relevant to colon carcinogenesis.

Wound Healing and Tissue Repair Effects

Propolis has been shown to stimulate various enzyme systems, cell metabolism, circulation and collagen formation, as well as improve the healing of burn wounds (Ghisalberti, 1979; Krell, 1996). These effects have been shown to be the result of the presence of arginine in propolis (Gabrys, et al, 1986). Propolis and aloe vera was found to be superior to standard wound treatment products in trials on mice (Sumano-Lopez, et al, 1989).

Gastro-Intestinal Effects

Propolis has been shown to inhibit the development of externally-induced stomach ulcers in rats (Aripov, 1988). Flavonoid components of propolis have also been shown to have this effect (Ciaceri and Attaguile, 1972).

Patients (138) suffering giardiasis were treated with propolis extracts (10-20%). In children, 52% showed a cure at the lower dose. In adults, the cure rate was the same as for tinidazole an antiprotozoan drug, at the 20% extract, and 60% versus 40% for Undazole at a higher concentration (30% propolis extract) (Mirayes, et a1$_1$ 1988).

Propolis was used to treat ulcerative colitis and Crohn's disease in a double-blind clinical trial in Denmark. Improvement was noted in patients with colitis, but no effect was shown against Crohn's disease (Stolko, et al. 1978).

Skin Infection Effects

The flavonoids and caffeic acid derivatives of propolis have been shown to be effective in inhibiting the growth of yeasts and fungi responsible for such skin infections as ringworm and athlete's foot (Metzner, et al, 1979

Anti-Inflammatory Effects
Studies on mice have shown that extracts of propolis have an anti-inflammatory effect due to the flavonoids and caffeic acid compounds (Mirozeva and Calder, 1996).

Anesthetic Effects
Propolis and some of its components produce anesthesia, which in some studies has been shown to be 3 times as powerful as cocaine and 52 times that of procaine, when tested in rabbit cornea (Ghisalberti, 1979). The anaesthetic effect has been shown to be produced by pinocembrin, pinostrobin, caffeic acid esters components in propolis (Paintz and Metzner, 1979).

The anesthetic effect may explain why propolis has been used for centuries in the treatment of sore throats and mouth sores. An anaesthetising ointment for dentistry using propolis has been patented in Europe (Sosnowski, 1984).

Effects on Immune System
Propolis has been shown to stimulate an immune response in mice (Manolova, et al, 1987). More recently, Japanese researchers have shown an extract of propolis to produce a macrophage activation phenomenon related to the immune function in humans (Moriyasu, et al, 1993). Propolis activates immune cells that produce cytokines. The results help to explain the ant-tumor effect produced by propolis.

Propolis has been shown to stimulate antibody formation in immunized mice. In a joint US-Polish study, spleen cells producing antibodies in mice administered a propolis extract were three times greater than controls. A second dose administered 24 hours later produced an even larger effect, although further doses reduced the effect (Scheller, et al, 1988).

Propolis was shown to increase antibody formation between 2-3 times that of controls in pigs vaccinated with "BUK-628" live Aujeszky's disease vaccine with and without addition of propolis. Antibody formation reached its maximum in 14 days, and antibodies could be detected for up to 330 days. Propolis also enhanced production of plasmacytes in the lympholdal tissue of the spleen and lymph nodes (Karandashov, et al, 1977).

Propolis has been shown to suppress HIV-1 replication and modulate *in vitro* immune responses, and, according to the authors, “May constitute a non-toxic natural product with both anti-HIV-1, and immunoregulatory effects” (Harish, et al, 1997).

Cardiovascular Effects
In mice, a concentrated extract of propolis has been shown to reduce blood pressure, produce a sedative effect, and maintain serum glucose (Kedzia et $a1_1$ 1988). $Dihydroflavonoids_1$ as contained in propolis, have been shown to strengthen capillaries (Roger, 1988), and produce antihyperlipidemic activity (Chol, 1991). Propolis has also been shown to protect the liver against alcohol (ethanol) and tetrachloride in rats (Coprean¸et al, 1986).

Adverse Effects
Propolis has been shown not to be toxic to humans or mammals unless very large quantities are administered (Ghisalberti, 1979). Some of its constituent flavones, eg., quercetin, might be mutagenic by the Ames test, but mutagenicity per *SE* for propolis has not been reported (Schmidt and Buchmann, 1992).

Contact dermatitis is a well-documented allergic reaction to propolis, with approximately 200 cases reported in the literature over the last 70 years (Hausen et al, 1987). Initial reports were made by beekeepers, which came into daily contact with the raw product. Allergic reactions are now also reported in the general population, due to the more wide-spread use of products containing propolis.

Dermatitis can be produced by skin contact with raw propolis, as well as propolis extracts and products containing caffeic acid and its derivatives have been identified as the major allergenic agent (Hashimoto, et al, 1988). Cinnamic acid derivatives have also been implicated (Scheller and Frosch, 1988). Dermatitis is relieved once the skin is no longer in contact with the propolis product. It is therefore recommended that with all preparations intended for human use, usage is ceased whenever there is an allergic reaction.
Very few other adverse reactions to propolis have been documented in the literature, and the product is considered generally not to be harmful (Schmidt and Buchmann, 1992). Rare cases of oral inflammation and ulceration, mouth edema (swelling) and stomatitis have been reported, however, because of oral ingestion of propolis (Hay and Grieg, 1990; Wanscher, 1978).

Commercial Usage of Propolis
Raw propolis is collected by beekeepers and sold in bulk to companies that refine the product and turn it into usable extracts. Main commercial uses of propolis are as a dietary supplement and therapeutic. Propolis is sold in tablets (singularly, or in combination with other substances such as pollen, royal jelly and non-hive products). In Japan, the use of propolis is permitted as a preservative in frozen fish (Irrell, 1996).

Tinctures and lozenges are popular treatment for sore throats, and tinctures are often used to treat Cuts, mouth sores and skin rashes. For internal use, 1-3mL does three times daily of a 1:10 tincture are typical, but higher doses can use used if necessary. Propolis tincture is normally diluted in water, producing a cloudy liquid. For external use, the 1:10 tincture is diluted in water, and used as a lotion or gargle (Bone, 1996).

Propolis is a stable product, but should nevertheless be stored in airtight containers in the dark, preferably away from excessive and direct heat. Propolis does not lose much of its antibiotic activity, even when stored for 12 months or longer. Propolis and its extract function as a mild preservative due to their antioxidant and antimicrobial activities and thus may prolong the shelf life of some products (Irell, 1996). Because of its antioxidant and antimicrobial activities, microbial contamination is not considered to be a problem with propolis, either in the raw form, or as extracts.

World propolis production is increasing substantially. Major producers include China, Brazil, US, Australia, Uruguay and Japan. Unfortunately, concentrations of lead above maximum allowable levels for food products have been found in propolis. Studies have shown that lead levels may be reduced by placement of hives away from areas with heavy air pollution and the use of oil based paints on hive parts (Alcici, 1996). Propolis destined for commercial use should be routinely tested for lead concentration. Brazilian propolis is of the highest quality available where Chinese propolis has been noted for excessive lead.

“Bee” warned however, that some individuals are sensitive to propolis and caution should be taken when beginning to consume it.

Beeswax

Beeswax is the major component of honeycomb. It is secreted in tiny flakes from the underside of the abdomens of worker bees, and molded into honeycomb. The wax of the western hive bee (Apis mellifera) differs from the beeswax produced by the Asian species of honey bee (Apis dorsata, florea, and cerana). Wax of the Asian species is called Ghedda wax and is less desirable than that of the western hive bee for international marketing purposes.

Beeswax is soft to brittle, with a specific gravity of about 0.95 and a melting point of over 60°C, and consists of at least 284 different compounds, mainly a variety of long-chain alkanes, acids, esters, polyesters and hydroxy esters. These include free cerotic acid (hexacosanoic acid, $CH_3(CH_2)_{14}COOH$), the ester of cerotic acid and triacontanol ($CH_3(CH_2)_{29}OH$), myricin (myricyl palmitate, $CH_3(CH_2)_{14}COO(CH_2)_{12}CH_3$), and hentriacontane, $CH_3(CH_2)_{29}CH_3$. Hentriacontane comprises 8-9% of beeswax, and its stability and impermeability to water contribute to the role it plays as a structural component. Although insoluble in water, beeswax can be dissolved in solvents such as carbon tetrachloride, chloroform, or warm ether. The exact composition of beeswax varies with location.

Beeswax is obtained, after removal of the honey, by melting the honeycomb, straining the wax to remove impurities, and pressing the residue to extract any remaining wax. The purified wax is then poured into molds to solidify. Avoiding direct heat through the melting of wax in water preserves color and quality of wax. The wax may also be bleached.

Pure beeswax is harder and has a higher melting point (64°C) than most other waxes. These properties make it more desirable for certain applications. Beeswax is primarily used industrially in cosmetics, pharmaceuticals, polishes, and candles. Uses for beeswax on a small scale include:

- lost-wax casting of metals

- wax printing and batiking of cloth
- polishes for wood and leather
- strengthening and waterproofing thread for sewing
- treatment of cracked hooves of domestic animals
- artificial fruit and flowers, and for modeling wax
- waxed paper

Honey Comb

Honey bees build comb for storage of honey to last them through the winter when the flowers they feed on are not available. The honeycomb is vertical with horizontal storage tubes, like a pile of unsharpened lead pencils carefully pressed together. Social wasps and hornets build vertical tubes. Honeycomb is two-faced with different tubes on each side; thus, an individual tube goes only halfway through the comb. Two of the six sides of the tubes are always vertical and each tube slants slightly downward toward the middle of the comb, which helps prevent the honey from running out as the worker bees fill it.

Honey bees engineer six-sided or hexagonal tubes because these use less wax for the volume of honey they hold. Each wall in the honeycomb serves two tubes that avoid the wasteful duplication of cylinders and most polygonal prisms. Only triangular or square tubes can also share all walls, but hexagonal tubes still use less wax for the amount of honey: 18% less than triangular tubes, 7% less than square tubes. Even more remarkable is the way the tubes meet in the middle of the comb. If you removed the honey and the wax where the tubes meet and peered through the holes, you would see that the tubes on opposite sides are offset: the center of a tube on one side is at the corner of tubes from the opposite side. The wax that separates the opposite tubes is not a single flat wall; instead, each tube ends in three rhombuses that come to a point. The three end walls of one tube serve as single walls for three adjacent tubes from the opposite side of the comb.

Honey Bee Venom

According to Greek mythology the infant Zeus, out of gratitude for the honey that sustained him, gave the honey bee its sting for defense. Because the bee abused this power, Zeus later decreed that the bee must die whenever the sting is used. Perhaps it is ironic that now we have developed the means to milk venom from bees and use this product in medicine. The collection and sale of bee venom is an increasingly popular although extremely limited enterprise.

Honey bee venom has been used medicinally for more than 2000 years in treating numerous types of acute and chronic afflictions. Currently physicians in China, Japan, Korea, Germany, Russia, South and North America, and some European countries are using honey bee venom (Bee Venom Therapy or BVT) in the treatment of chronic inflammatory diseases. Bee venom has a wide range of medically important and pharmacologically active compounds. Several of them already have been identified,

notably melittin and apamin, with outstanding therapeutic potential for cancer, sleep disorders, learning and memory enhancement, Parkinson's disease, HIV and AIDS associated dementia, schizophrenia, and novel non-viral vector development for gene therapy. In the United States many physicians are also using BVT with some success in the treatment of multiple sclerosis, Lyme disease, autoimmune diseases, psoriasis, epilepsy, asthma, and certain types of cancer.

The world scientific literature contains more than 1500 articles on the medicinal value of BVT. The French and Russian equivalents of the N.I.H. have been involved in clinical studies of honey bee venom, and the U.S. Army has research extensively BVT. In April 1993, the Nanjing Institute of Biochemical Pharmacy in China not only noted the anti-inflammatory properties of bee venom, but also demonstrated its analgesic effects.

Traditionally, honey bee venom has been administered with live bees by stimulating them to sting in the affected area or on an acupuncture point. Current day variety of honey bee venom products includes: injectable liquid venom, creams, liniments, ointments, embrocations, and oral homeopathic preparations as liquids, tablets, or capsules. Practitioners may choose the most suitable application for the condition being treated and the characteristics of the patient.

BVT is most effective when it comes from bees during the late spring to early fall season. This is when bees have an abundant source of pollen to produce potent venom. Their venom during the winter period is less potent. Unlike many other insect types of venom, honey bee venom is water soluble, not fat soluble, and so must be injected into moist tissue to be effective. It is hemorrhagic, unlike viper snake venom, which is a coagulant. Honey bee venom is a mixture of histamine, pheromones, enzymes, peptides, amino acids and other acids.

In short, honey bee venom contains anti-inflammatory properties, is mildly cytotoxic, and has the contradictory effects of inhibiting the nervous system, while stimulating the heart and adrenal glands.

Next to the effect of a live honey bee, injectable venom solution is considered to be a standard method to administer BVT. The injectable venom solution is prepared from pure honey bee venom (Apis Venenum Purum). The solution is administered just under the skin to imitate the effect of a bee sting and each injection is equivalent to or is less than the average dry venom sac content of a honey bee. Another popular way of administering BVT is with topical creams and ointments applied to the affected body part. In Europe and China BVT cream is applied utilizing electrophoresis or ultrasonophoresis.

Bee Venom Reactions and Sensitivity

For most individuals, normally some redness and swelling will result from the sting, but this usually resolves in a few hours. It is also common that is an itching sensation around the sting. A mild euphoria and local warmth usually results. However, in the

allergic individual, a more long lasting and severe reaction will occur. A mild reaction will include intense redness, swelling spanning two joints, itching and pain all occurring within minutes. More severe reactions include generalized swelling and itching, faintness, sweating, a pounding headache, stomach cramps or vomiting, a feeling of impending doom, a tight chest or choking sensation with swelling of the throat and in extreme cases anaphylactic shock with death resulting.

Generally side effects of bee venom therapy are limited, since the inflammation, swelling, and itching, etc. are desired effects. The risk of an anaphylactic allergic reaction to bee venom is rare but real. Hence, it is essential to have a bee sting allergy kit on hand. Most "bee" sting allergic reactions are to yellow jackets or wasps. There is no cross allergy between wasp or yellow jacket venom and honey bee venom. Because of their vegetarian nature the peptides from honey bees are different and less toxic than their carnivorous cousins. It is estimated that honey bee stings account for less than 5% of all adverse stinging insect reactions.

Honey bee venom may have many different effects on the human body. This is based on the potency and total effect of the bee venom. Generally, a person who is not hypersensitive to bee stings can tolerate one to five stings at a time. This is followed by minimal local symptoms accompanied by swelling, redness and itchiness of the skin. Initially, the symptoms are little painful but later change to a pleasant and warm sensation. Fifty to one hundred stings can cause a cramp, temporary shortness of breath, the skin turns blue or rapid pulse and symptoms of temporary paralysis follow. Two hundred or more bee stings can cause paralysis of the respiratory system. Some people can tolerate more than 1,000 stings. In one case it was noted that an unfortunate individual received 2,243 stings and stayed alive. Many adults have received a thousand stings and survived. A rough estimate would be that the LD50 on adult humans is about 1,000 to 1,100 stings. On the other hand, we know from reports that 100 to 300 bee stings have had a fatal effect on some unlucky individuals. However, to a person who is hypersensitive to honey bee venom, only one sting can cause a serious or fatal reaction (very rare).

If a hypersensitive person is accidentally stung, it is important to remove the stinger as early as possible. This is best done with a pair of tweezers to not squeeze the venom sac of the stinger. After it is removed, lay the victim down on their back and elevate their feet. A tourniquet can be very useful if applied immediately and briefly to a limb. Applying ice to the sting will also reduce blood flow to the areas and decrease the spread of venom.

For these severe reactions, preloaded adrenalin syringes (Ana-Guard or Epipen) are available for emergency use. Three to six puffs of an adrenalin inhaler (Medihaler-Epi) will relieve chest tightness and swelling of the throat. With less severe reactions, antihistamines may be administered by injection or given orally. A rapidly acting antihistamine such as Phenergan has been proven helpful. Cortisone is also very effective, but takes a few hours to act.

Apis Venenum Perum (Pure Honeybee Venom)
Synonyms:
Latin --- Apis Virus, Apisinum, Apium Virus
French --- Venin d'abeille
German --- Bienengift
Hungarian --- Méhméreg
Italian --- Veleno d'api
Spanish --- Veneno de Abeja

Venom Collection and Preparation
As previously mentioned honeybee venom is synthesized in the venom glands of worker and queen bees and stored in their venom sacs. During the stinging process it is expressed through the sting apparatus. Usually, bee venom is collected during the peak of autumn when the bees' venom sacs are full of quality venom, so that the venom is of high quality when it is reconstituted.

The venom is usually gathered by means of electric shock stimulation. Bees come into contact with a collector frame that is covered with a wire grid, receive a mild electrical shock causing them to release their venom. The venom is allowed to air dry and is then gathered and processed**.** Approximately a minimum of 4000 bee stings is needed to produce 1 gram of bee venom. Traditional collection methods used up to until now usually killed the bees. However, the bee venom collected by the electro-stimulant method, does not kill the bee.

Now at least four qualities of bee venom can be collected in this manner. Bee venom is a colorless liquid that dries to a powder and the crystallized venom color ranges from white to brownish yellow. The darker coloration is the result of contamination of the venom and oxidation of its constituents.

The pure whole dried and the whole dried bee venom are most commonly used preparations. Pure whole dried bee venom is white in color, has a crystallized powdery appearance, is free of contaminants and is known as Grade I venom. Whole dried bee venom is of brownish yellow color and may be contaminated with foreign materials. It is referred to as Grade II venom.

Honeybee Venom Solutions Prepared from Grade I Venom

Product	Volume	DVSE/vial	Appearance
VeneX-5	2.8 ml	14	Water clear
VeneX-10	9.0 ml	90	Water clear
VeneX-20	12.5 ml	250	Water clear

VeneX-20

Most Recommended

Honeybee Venom Solution Prepared from Grade II Venom

Product	Volume	DVSE/vial	Appearance
BVS-20	12.5 ml	250	Water clear to yellowish

Injectable Application and Methodology of Bee Venom
(Using Grade I Venom VeneX-20)

Before injection, it is essential to have epinephrine and Benadryl on hand in the rare case of allergic reaction. Tell the patient the usual discomfort associated with the administration of bee venom includes mild pain, itching, swelling, inflammation and redness. Symptoms like redness, swelling and itching are considered desired effects of BVT showing the appropriate response of the patient to the venom. The pain may somewhat be avoided with the addition of 1% procaine.

Procure the solution VeneX-20. This is a solution that contains pure whole dried bee venom. VeneX-20, in the amount of 0.05 ml equals one (1) of a venom sac equivalent (bee sting equivalent) and 0.1 ml equals two (2) venom sac equivalents. Before use, remove from refrigeration, allowing 25 to 30 minutes for the solution to reach room temperature. Cleanse the exterior surface of the closures of the vial with a suitable disinfecting agent.

Using a tuberculin syringe with a removable 27-gauge, ½" long needle, draw up 0.5 ml of 1% procaine, then add 0.5 ml of Venex-20 to create an equal mixture of both anesthetic and venom solution. (A 1% xylocaine may be used if preservative-free buffered procaine is not available) This is equivalent to 1.0 ml of bee venom and anesthetic mixture or 10 bee stings. This 1.0 ml of mixture-solution is equal to 10 bee stings. Hence, this 1.0 ml will provide ten (10) 0.1 ml injections and each injection will equal one (1) bee sting. Remember 0.05 ml of VeneX-20 equals one (1) bee sting!

At the first session, it is imperative to test for an allergy to the venom. A test solution is prepared using the formula below. Administer the test solution into a tender spot and wait 20 to 30 minutes for any allergic symptoms to develop. If no symptoms develop then begin the therapy. Again, keep Anakit or Epipen and benadryl on hand!

Use ether acetone or soap with warm water to cleanse the skin. Do not use alcohol, iodine, potassium permanganate, potassium sulfate, halogen elements, chlorine and bromine, because they will cancel the effects of the bee venom. The needle is changed to a 30 gauge, 1" length and the bevel is placed at a 15-degree angle. Injection is strictly intradermally (between the skin layers) or subcutaneous (just under the skin, beyond

the sensory nerves of the skin) to imitate a bee sting. Inject 0.1 ml of mixture into the chosen point.

Length of Venom Treatment

Plan ahead how many injections will be needed, and which sites or acupuncture points will be used. At first begin with only one to three injections. After several sessions, more injections may be added depending on tolerance. In most circumstances, 3 to 10 points at a time may be chosen. This process may be repeated every 2 to 3 days as necessary to achieve the desired effect. Never ever administer more than twenty injections at one sitting (20X 0.05 ml of VeneX-20). Be (bee) conservative. A more chronic problem, such as rheumatoid arthritis, might require several stings at sessions up to three times a week for up to three months. For certain illnesses and circumstances, it may be necessary to educate the patient to self-administer the injection. If more than 10 to 14 days have elapsed since the previous session, retest for sensitivity and begin with three (3) to four (4) stings per session.

The patient should strictly avoid alcohol ingestion during the course of treatment. Alcohol cancels the beneficial effects of BVT. In fact, reactions to BVT while intoxicated have been reported.

Acupuncture with Venom (Api-acupuncture)

Acupuncture has been practiced in ancient China, Japan, Korea, Sri Lanka, and numerous other Asian countries for about 3,000 years as a traditional form of medicine. Its efficacy in the management of numerous illnesses as well as severe chronic pain is well documented. Though its primary use in the United States is for pain management, its use in Asia is quite diverse and for many types of illnesses. The fact of its world widespread use today attests to its validity as an effective form of treatment in a variety of disorders. It is taught in many universities throughout the world and practiced widely in many modern medical centers as an adjunct to Western methods of treatment. It is often used where Western medicine meets its limits: in the treatment of various forms of paralysis, internal metabolic disorders, infertility, chronic pain, drug-addictions, etc. The World Health Organization now recognizes that acupuncture is beneficial for more than 150 types of diseases.

Acupuncture consists of inserting a very fine needle at specific points in the skin. Recently other means of stimulating these acupuncture points have evolved, such as laser, electric stimulation, magnets, seeds, finger pressure, and substance or remedy injection. Acupuncture points follow meridians (pathways of vital energy or Qi) and have been shown that when stimulated transmit electrical impulses via the spinal cord and the brain to the diseased area. The central nervous system controls most processes in the body. Modern research has also shown that the stimulation of certain acupuncture

points produces the release of chemical substances from the central nervous and endocrine systems. Some of these substances such as enkaphalins and endorphins are 5,000 to 50,000 times more powerful than morphine. The injection of herbal substances, vitamins and homeopathic remedies into acupuncture points is becoming better known.

The stinging of acupuncture points with honey bees has been traditionally used in China and Japan for centuries. A society for api-acupuncture was formed in 1980 in Japan. In the following years, many reports of experiences and successes in api-acupuncture appeared (in Japanese) in Honey Bee Science (e.g. Ohta, 1983 and Sagawa, 1983). In the Republic of China, bee venom therapy is combined with knowledge of acupuncture by many hospitals and physicians.

Honey bee venom may be injected with a 30-gauge needle inserted intracutaneously or subcutaneously into the acupuncture point following standard acupuncture principles. Within the first treatment, no more than 0.1 ml of VeneX-20 solution (two VSE) is used in a syringe together with an equal amount of 1% preservative free procaine solution. Always administer a test injection first! The amount of venom mixture can be gradually increased. If necessary to reduce the stinging sensation during the injection the amount of procaine may be increased (0.05 ml Venex-20 with 0.5 ml 1% preservative free procaine = one VSE).

NOTE:
No benefit has been reported when injecting venom intramuscularly or into any structure deeply below the skin, such as autonomic ganglia, joints, deep trigger points, or intravenously.

Another method commonly used: an acupuncture needle is dipped into the venom/ procaine mixture and inserted into the acupuncture point (horizontally/superficially), or a drop (one VSE) of the venom/procaine mixture is applied to the skin at the acupuncture point and the needle is inserted through the venom remedy. In both cases only a minute amount of bee venom is administered.

For best results chose only one to three local acupuncture points on the afflicted meridian. It cannot be overstated that it is important to ascertain the meridian or meridians that are affected and choose points on those meridians depending on the inflammatory or degenerative condition.

ApiAcupuncture Indications

As previously mentioned bee venom (apitherapy) primarily is used to treat a variety of autoimmune diseases, with recent usage for immunotherapy of bee sting allergic patients. (Immunotherapy use will not be considered in this text.) Venom apitherapy has been particularly successful with individuals suffering from rheumatoid arthritis, gout, and multiple sclerosis, but a variety of other immune disorders including scleroderma and asthma have been successfully treated.

Complete acupuncture treatment protocols are beyond the scope of this article. A basic knowledge of traditional Chinese medicine is crucial for the thorough understanding and effective application of acupuncture. The text *Chinese Acupuncture and Moxabustion* by Foreign Languages Press, Beijing is highly recommended. For those versed in acupuncture theory and technique the following are a few specific examples of conditions treatable with venom injection into acupuncture points:

Application of Honey Bee Stinger Therapy

Most practitioners much prefer using the injectable venom to bee stingers. However, many individuals still prefer to apply bee venom by using a live bee to sting at a specific site, and repeating this procedure over a period of time. Actually, the application of a carefully removed stinger with fine tweezers into a specific acupuncture point is still popular in Japan, Korea and China. The venom of one sting is applied into 3 - 6 acupuncture points, which provides a safe way of practicing bee venom therapy.

A person with poor vision or who has a distinct fear of honey bees may have problems in removing the bee stinger with tweezers. In this case, prepare two sheets of toilet tissue and fold them in halves. Pick up a honey bee with tweezers and place the soft toilet tissue to the bee's tail, pressing the tail with slight force. While pressing the tail, the honey bee stings the tissue. Then, press and kill the honeybee and remove the bee stinger on the tissue.

Removing the stinger in this way allows the tissue to absorb and discard the initial strong bee venom and then the secondary venom from the bee venom reservoir is actually used to the human skin.

Picking up the honeybee from the plastic honeycomb

Honey bees by mail come in plastic combs. To remove the bee, push one side of the plastic honeycomb gently, and allow an entrance of approximately 5 mm. Then, using a pair of tweezers placed inside the honeycomb, remove one honeybee. Like injectable applications, the number, sites, and frequency of the stings depend on the patient and the problem. A simple tendonitis might just take a few stings, perhaps 2-3 at a session for 2-5 sessions. A more chronic problem like arthritis can take 2-3 times per week, several stings at a time, for 1-3 months. Multiple sclerosis takes months to respond, though sometimes patients feel more energetic after a few times. Many MS and Lyme disease patients who use honeybee venom claim that one must keep it up 2-3 times per week for 6 months in order to give it a full trial.

Honeybee Venom Creams

To make a cream, bee venom is mixed in a water-in-oil or oil-in-water base vehicle, which may have some added ingredients to help soften the outer layer of skin increase local blood circulation and cause mild abrasions to enhance the absorption of venom.

This is a painless way to administer bee venom and can be carried out by the patient. Its efficacy is less than that of injectable venom solution.

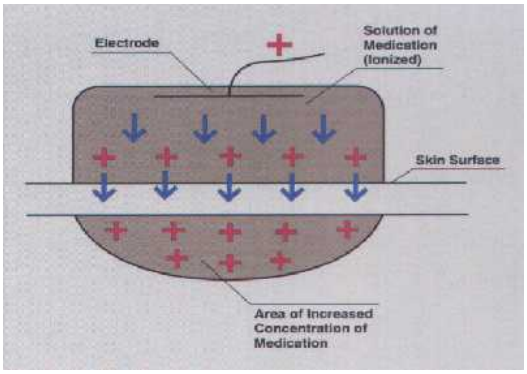
Honeybee venom creams have been used successfully in several cases of rheumatoid polyarthritis. The results have shown an important reduction in muscular pain, sciatic pain, nerve pain, neuromyalgias and intercostal and bronchial nerve pain. Venom creams are also useful for daily application to scars.

There are numerous honeybee venom creams on the market. The following creams are the most commercially available.

Product	**Weight**	**Country**
Forapin E Salbe	50 g & 100g	Germany
ApiMel	56 g	USA
Apicure	30 g	New Zealand
Bee V Balm	30 g	New Zealand
ApiVENZ	25 g	New Zealand
Venex	30g	Canada
Apisarthron	50 g	Germany

Creams Applied with Iontophoresis and Ultrasonophoresis

Bee venom creams may be applied topically on the affected area or by electrophoresis and ultrasound. These methods are known as a safe and effective means of absorption of medicinal substances. Iontophoresis is a process that moves particles between electrodes through a liquid or gel utilizing an electrical current and is an effective method of delivering bee venom to localized tissue. The diagram below illustrates the basic mechanism. Like electrical charges repel. Therefore, application of a positive current will drive positively charged drug molecules away from the electrode and into the tissues; similarly, a negative current will drive negatively charged ions into the tissues.



Ultrasonophoresis moves particles through a gel using vibration of an ultrasonic wave or sound with frequency ranges inaudible to humans. The venom cream is first tested in a small area for sensitivity. If no adverse reactions occur after 30 minutes, the cream is applied to the area selected and iontophoresis or ultrasononophoresis is applied. The frequency of treatments depends on the severity and chronicity of the arthritis or affliction. This is an excellent method in application of venom to scar tissue.

Venom Properties and Composition

Honey bee venom contains more than 60 components that are uniquely pharmacologically active. The following table expresses the composition of venom from a honey bee worker as stated in the findings of two separate studies (Dotimas and Hilder 1987 and Shipolini 1984). These components were measured as a percentage of dry venom, with water makes up approximately 88% of venom before drying.

Class of Molecules Component % of Dry Venom (1) % of Dry Venom (2)

Class of Molecules	Component	% of Dry Venom (1)	% of Dry Venom (2)
Enzymes	Phospolipase A2	10-12	10-12
	Hyaluronidase	1-3	1.5-2.0
	Acid phosphomonoesterase		1.0
	Lysophopholipase		1.0
	a -glucosidase		0.6
Other Proteins and	Melittin	50	40-50
Peptides	Apamine	1-3	3
	Mast Cell Degranulating Peptide (MCD)	1-2	2.0
	Secapin	0.5-2.0	0.5
	Procamine	1-2	1.4
	Adolapin		1.0
	Protease inhibitor		0.8
	Tertiapin *	0.1	0.1
	Small peptides (with less than 5 amino acids)	13-15	
Physiologically Active	Histamine	0.5-2.0	0.6-1.6

Amines	Dopamine	0.2-1.0	0.13-1.0
	Noradrenaline	0.1-0.5	0.1-0.7
Amino Acids	t -aminobutyric acid	0.5	0.4
Sugars	Glucose and fructose	2	
Phospholipids		5	
Volatile compounds		4-8	

The most intensely studied components are: melittin, apamin, peptide 401, adolapin, protease inhibitors, and phospholipases.

Melittin

Melittin is the most prevalent biologically active substance of honey bee venom. It stimulates the hypophyseal-adrenal system and produces cortisol. It is 100 times more potent than hydrocortisone (Couch, 1972; Knepel *et al*., 1987; Vick *et al*., 1972, 1975). Melittin also stabilizes the lysosome cell membrane to protect against inflammation (Shkenderov et al., 1986). It is an inhibitor of protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II, myosin light chain kinase and $Na+/K^{+}$-ATPase (synaptosomal membrane), with an IC50 of 1-4 μM; cell membrane lytic factor. Melittin has also been shown to inhibit neutrophil superoxide and hydrogen peroxide production. This probably accounts for some of the anti-inflammatory activity of bee venom, since oxygen radicals contribute to inflammatory tissue damage.

Melittin is quite a short peptide (only 26 amino acids long). Its underlying structure is helical but its overall shape is that of a bent rod. In the venom sac of the bee, melittin adopts a tetrameric conformation. The tetramer forms much in the same pattern as a baker would arrange pastry on a hot cross bun. As a result, the core is made up of apolar residues that are completely shielded by a hydrophilic coat.

The Tetrameric Conformation of Melittin

The monomeric conformation seems to be adopted once melittin is injected via a bee sting and hits cell membranes. This is where melittin is so interesting. It is a powerful cell-lytic agent. It binds rapidly to erythrocytes bringing on the release of hemoglobin in the extracellular medium. Seeing that there are about 1.8x10 (to the power of) 7 binding sites for melittin per erythrocyte, it is likely that the site of interaction is the membrane lipid rather than specific receptors. Lysis occurs when the organization of a lipid bilayer is perturbed.

Though, to date, there is little direct evidence on the molecular mechanism of hemolysis, it is thought that lysis occurs by a colloid osmotic mechanism which results in the formation of melittin-induced lesions or “pores”. In fact, melittin may interact with

membranes in different ways depending on the lipid composition of the bilayer; in the event of channel formation, the tetrameric conformation of melittin seems to be required. The scientific interest in melittin is obvious. The assimilation of certain types of drug could be largely facilitated if coupled with melittin.

ABSTRACT

A matrix metalloproteinase 2 cleavable melittin/avidin conjugate specifically targets tumor cells in vitro and in vivo.

Holle L, Song W, Holle E, Wei Y, Wagner T, Yu X.

Department of Biological Science, Clemson University, Clemson, SC 29634, USA.

Extracellular matrix breakdown as well as increased expression in cancer cells and tumor microvascular endothelial cells make matrix metalloproteinase 2 (MMP2) an attractive target for cancer treatment. By taking advantage of MMP2's properties, an MMP2 cleavable melittin/avidin conjugate was designed. Melittin alone is extremely toxic to cells and induces immediate cell lysis, but becomes inactive when coupled with avidin. The incorporation of the MMP2 target sequence into the peptide was used as a means for targeting tumor cells. In vitro, the melittin/avidin conjugate showed strong cytolytic activity against cancer cells with high MMP2 activity; DU 145 prostate cancer cells and SK-OV-3 ovarian cancer cells. The conjugate exhibited very little cytolytic activity against normal L-cells that displayed low MMP2 activity. These data demonstrate the MMP2 specificity of the melittin/avidin conjugate. In vivo, the size of tumors injected with the melittin/avidin conjugate was significantly smaller as compared to untreated tumors. Therefore, due to its tumor targeting capabilities as well as its cytolytic properties in vitro and in vivo, the melittin/avidin conjugate displays the potential for use in cancer therapy.

Other Cancer References:

- Ginsberg-N-J. Dauer-M. Slotta-K-H.T1 Melittin used as a protective agent against x-irradiation. Nature. 1968 Dec 28. 220(174), p. 1334.
- Hait-W-N. Grais-L. Benz-C. Cadman-E-C.TI Inhibition of growth of leukemic cells by inhibitors of calmodulin: phenothiazines and melittin, Cancer-Chemother-Pharmacol. 1985. 14(3). P 202-5.AB Calmodulin, a ubiquitous calciumbinding protein, has recently been shown to play an important role in cellular proliferation. The calmodulin inhibitors melittin, trifluoperazine, and chlorpromazine inhibited the growth and clonogenicity of human and murine leukemic cells, and their potency reflected their activity as inhibitors of calmodulin. Melittin, which is a far more potent inhibitor of calmodulin activity, was also a more potent inhibitor of cell growth and clonogenicity. The less active phenothiazine metabolite, chlorpromazine sulfoxide, had much less potent cytotoxic activity.
- Killion, JJ. Dunn-J-D.TI. Differential cytolysis of murine spleen, bone-marrow, and leukemia cells by melittin reveals differences in membrane topography. Biocbem-Biophys Res-Commun. 1986 Aug 29. 139(l), p. 222-7. AB L1210 leukemia cells are 2-4 fold more sensitive to the cytolytic effects of melittin, the

membrane-active toxin of bee venom, than normal DBA/2 mouse spleen and bone-marrow cells.

- Gerst, J.E., Salomon-Y.TI. Inhibition by Melittin and fluphenazine of melanotropin receptor function and adeylate cyclase in M2R melanoma cell membranes. Endocrinology. 1987 Nov.121(5), p. 1766-72.

Scientists at the Oncology Research Centre at Prince of Wales Hospital in Australia claim to have killed cancer cells in a test-tube using synthesized melittin, which destroys cells by creating a hole in their outer membranes. They further claim they have modified the structure of the melittin molecule by removing the part that causes allergic reactions in some patients, while maintaining its cell-killing ability.

Apamin

Apamin is a small, basic, 18-amino acid peptide toxin with 2 disulfide bridges that has the ability to cross the blood brain barrier. Apamin also blocks a class of Ca^{2+}-activated K^{+} channels, thus potentially enhancing nerve transmission. It works like melittin to produce cortisol (Vick and Shipman, 1972), and inhibits the complement system, C3, which is involved in inflammation (Gencheva et al., 1986). Apamin is not only an anti-inflammatory, but also acts as a mood elevator. Hence, apamin is a plausible explanation as to why people feel better after being stung.

ABSTRACT
Ikonen S, Riekkinen P.
Effects of apamin on memory processing of hippocampal-lesioned mice.
Eur J Pharmacol 1999;382:151-156
We investigated the effects of acute i.p. injections of the Ca(2+)-dependent K(+) channel blocker, apamin, on water maze spatial navigation and radial arm maze performance in mice with partial hippocampal-lesions. In the radial arm maze, apamin 0.06 and 0.2 mg/kg dose-dependently reversed the lesion-induced defect. In the water maze, apamin 0.2 mg/kg alleviated the defect, but a lower dose 0.06 mg/kg was ineffective. At a higher dose, 0.4 mg/kg, apamin impaired the water maze performance. These results suggest that Ca(2+)-dependent K(+) channel blockers can alleviate the spatial reference memory and working memory impairment induced by partial hippocampal lesions.

ABSTRACT
Ikonen S, Schmidt B, Riekkinen P Jr.
Apamin improves spatial navigation in medial septal-lesioned mice.
Eur J Pharmacol 1998;347:13-21
We investigated the effects of acute i.p. injections of the Ca2+-dependent K+ channel blocker, apamin, on water maze spatial navigation, Y-maze and passive avoidance behavior in intact and medial septal-lesioned mice. Apamin 0.02, 0.06 or 0.2 mg/kg (i.p.) administered 30 min before or immediately after the training did not affect the performance of intact mice. Apamin 0.02 or 0.06 mg/kg (i.p.) administered immediately

after the daily training did not affect the performance of medial septal-lesioned mice. Apamin 0.02 and 0.06 mg/kg (i.p.) administered 30 min before daily training reversed the navigation failure present in medial septal-lesioned mice during the initial and reversal learning stages of the water maze task. Apamin had no effect on the cognitive performance in Y-maze or passive avoidance tests. The results indicate that blockade of Ca2+-dependent K+ channels may facilitate acquisition of spatial navigation performance, but has no effect on consolidation, inhibitory avoidance and spontaneous alternation behavior in mice.

Peptide 401, or MDC Peptide

Peptide 401, or MDC peptide, blocks the arachidonic acid and inhibits prostaglandin synthesis (Hanson et al., 1974; Neubould, 1963; Surfer *et al*., 1973) In July 1990, Ziai, Russek, Wang, Beer, Blume from the American Cyanamid Company, Pearl River, New York, commented that peptide-401 displays striking immunological and pharmacological activities. They also demonstrated that MCD significantly lowered blood pressure in rats. Peptide 401 was also found to be effective on edema (Billingham *et al*., 1973).

Br J Pharmacol. 1990 Feb;99(2):350-4.
Anti-inflammatory activity of bee venom peptide 401 (mast cell degranulating peptide) and compound 48/80 results from mast cell degranulation in vivo.
Banks BE, Dempsey CE, Vernon CA, Warner JA, Yamey J.
Department of Physiology, University College London.
1. The relationship between the anti-inflammatory activity of the bee venom peptide 401 in the carrageenin-induced oedema of the rat hind paw and its mast cell degranulating activity has been reinvestigated. 2. Mast cell degranulation caused by compound 48/80 (10 mg kg-1) or by allergen challenge in rats sensitized to Nippostrongylus brasiliensis also suppressed rat hind paw oedema in the same test. 3. The anti-inflammatory activities of peptide 401 and compound 48/80 were partially suppressed by pretreatment of rats with mepyramine and methysergide, at doses (2.5 mg kg-1) that completely suppressed skin reactions to these mast cell-derived amines. Pretreatment of rats with compound 48/80 also suppressed the apparent anti-inflammatory actions of peptide 401 and of compound 48/80. 4. Injection of peptide 401 together with carrageenin increased the inflammatory response in the rat hind paw. 5. The anti-inflammatory activity of peptide 401 and of compound 48/80 in the carrageenin-induced swelling of the rat hind paw arises from mast cell degranulation in vivo.

Adolapin

In 1982, Bulgarian researchers Shkendrov and Koburova isolated a peptide in bee venom called adolapin and showed that it had anti-inflammatory and analgesic properties. Adolapin inhibits the microsomal cyclooxygenase. It is 70 times stronger than indomethacin in animal models (Shkenderov *et al*., 1986). It also inhibits platelet lipoxygenase, which involves hydroperoxyeicotetranonic acid (HPETE) and leukotriens

(Koburova *et al*., 1985), as well as inhibiting thromboxane (TXA2) and prostacycline (PGI2), which are activated during inflammation (Shkenderov *et al*, 1986). By inhibiting cyclooxygenase, adolapin has an analgesic effect.

Protease Inhibitors

Protease inhibitors inhibit carrageenin, prostaglandin E1, bradykinin, and histamine induced inflammations; they also inhibit chymotrypsin and leucine-aminopeptidase (Shkenderov, 1986).

Phospholipases

Like snake venom, bee venom has phospholipases (12%) as major active components, which are freed from the lysosomes of phagocytes in the course of inflammation (Zurier *et al*., 1973). These phospholipases can cleave phospholipids of the cell membrane to create arachidonic acid, which is converted into prostaglandins. In fact, a phospholipase A2 inhibitor was able to block the inflammatory pathway in acute and chronic situations (Garcia-Pastor *et al*., 1999. In addition, honey bee venom contains lecithinase converts lecithine to lysolecithine (or phospholipase B), which breaks down the membranes of blood cells, and hyaluronidase (3%) acts as a spreading factor, by breaking down hyaluronic acid, a polysaccharide interstitial fluid in connective tissue.

Other Beneficial Substances

Other unique substances, such as Compound X, Hyaluronidase, Phospholipase A2, Histamine, and Mast Cell Degranulating Protein (MSDP), are involved in the beneficial effects of bee venom. The main amino acids in bee venom are cysteine and methionine, both of which are sulphur bearing. Sulphur is important in inducing cortisol release from the adrenal glands. Finally, the venom also contains measurable amounts of the neurotransmitters dopamine, norepinephrine and serotonin.

Unique Physiological Effects of Venom

Antibacterial, Antifungal, Antiradioactive Activity

Schmidt-Lange (1941), Ortel (1955), and Fennell *et al*. (1968) reported that bee venom has a strong anti-bacterial and anti-fungal effect as well as a radioprotection effect (Ginsberg *et al*., 1968; Kanno *et al*., 1970; Shipman et al, 1967, 1968).

Conclusions

People's lives would be greatly different without the benefits provided by honey bees, and it is unlikely that we would be as culturally evolved as we are without them. We would not have fruits, vegetables, and plants that depend on their pollination. Their miraculous products - honey, propolis, royal jelly and venom - provide us with a

medicinal treasure chest. Without them we would know much less about genetics and other areas of biology. Scientists and poets alike are continually inspired and fascinated by the habits of these divine insects.

To make a prairie it takes a clover and one bee,
One clover, and a bee . . .
— Emily Dickinson

The information in this monograph is intended for informational purposes only, and is meant to help users better understand health concerns. Information is based on review of scientific research data, historical practice patterns, and clinical experience. This information should not be interpreted as specific medical advice. Users should consult with a qualified healthcare provider for specific questions regarding therapies, diagnosis and/or health conditions, prior to making therapeutic decisions.

For more information, visit www.brmi.online.