A remarkable hive product:

PROPOLIS

Scientific data and suggestions concerning its composition, properties and possible use in therapeutics

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PROPOLIS

Scientific data and suggestions concerning its composition, properties and possible use in therapeutics

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FOREWORD

The issue of a beekeeping book or having in any way connection with apiculture is now also the interest of specialists others than professionals. This is due to the new direction of people of our epoch towards a more complex use of the natural products.

Bees offer a large scale of processed products used in food industry and also used in order to keep up man's health.

The anthology of texts entitled "Propolis" appears in the series of APIMONDIA Publishing House issues which refer to scientific works on apicultural themes issued in booklets, reports presented to some international symposia or in the Scientific (Biennial) Bulletin of APIMONDIA. The present book gathers the reports on propolis presented in the symposia of apytherapy organised by the International Federation of Apicultural Associations, held in Bratislava in 1972, Madrid in 1974 and Bucharest in 1976 as well as articles issued in the last years in apicultural literature, in biochemistry, biology, technology etc.

This book is among the publications referring to the origin, composition and processing of hive products and also to their use in everyday life. Also, in the Publishing House of APIMONDIA International Institute for Beekeeping Technology and Economy, the following works were translated and recently published: "Honey and Other Natural Products" *by the American doctor D. C. Jarvis and* "Pollen" *by the French engineer Alin Caillas. Both represent a long study and experimentation performed by the authors who clarified some aspects connected with the structure and conversion of hive products as well as of other natural products.* "Apitherapy to-day" *is a third one, aiming also to provide ample information for the consumer's use. Thus, in such series with monographic character, this volume about propolis is included, being considered a hive product less studied and thus less known by scientists. It is nevertheless highly appreciated due to its beneficial applications both in the life of the bee colony and in popular medicine.*

The APIMONDIA Publishing House wanted also to completely present this very old and common product of empirical practice, product which is now recommended with authorized scientific indications.

We could thus explain the presence in the pages of the book of some heterogenous materials. By publishing them in their original form we understood to give the producers on the one hand and the practitioners in the domain of chemistry, pharmacy, medicine on the other hand, a document and information which should cast a view on the present stage of knowledge concerning propolis as well as its broad prospectives.

We are convinced that the publishing of this book will contribute to the spread of theoretical and practical knowledge about propolis. In the same idea we belive that informmations and documentary references which our publication contains will stimulate the approach of further studies and research work meant to clarify the less known aspects of propolis — natural bee product, which helped to a larger use and efficiency for people.

President of APIMONDIA
Prof. Dr. Eng. V. HARNAJ

I. GENERALITIES

PROPOLIS

A. CAILLAS
FRANCE

All beekeepers know propolis as a by-product of the hive : but this is not entirely correct for as we will see and whose origin use shall learn with this substance the bees glue all parts of their shelter that are more or less moveable, stop up gaps and consolidate everything that appears to be less solid.

The name "propolis" is the result of combining two terms, Latin and Greek : *pro,* which means "in front of" or "before" and *polis* which means "fortress".

In regions where the temperature is quite high and in order to protect themselves from some of their enemies, honey bees build genuine barriers of propolis behind the entrance to the colony, and fortifications means to hinder the passage of enemies.

Propolis is also used by the bees for gluing the frames one to another, to the great inconvenience of beekeepers when manipulating their colonies. It is also used for varnishing the interior walls of the hive.

It has a two-form origin :

1) An internal origin. According to the German researchers KÜSTENMACHER & PHILLIPP and WECK and some others, propolis might be a resin residue coming from the first phase of pollen digestion in a small organ placed between the sac and the lower gut.

All cells, and especially the newly built ones, are varnished with this internal propolis before the queen lays eggs in them : this results from the experiments of Dr. BRUNNING.

The greatest quantity of propolis produced by the bees seems to have this origin.

It can be easily recognized under the microscope because of the pollen grains it contains.

2) The second origin of propolis is external. In former times little was known about it. Bee researchers believed that the forager bees harvested propolis only from tree buds, particularly from poplar and alder. It has been found however that they harvest it also from other trees since propolis has been found in hives in places where there are neither alders nor poplars. It is well known by all apiarists that the hives placed in forests have more propolis in them than those on the plain.

Composition

Propolis, not being a determined substance, has no chemical formula.

As the author of this paper demonstrated in a lecture in the Academy of sciences in Paris, delivered on November 26, 1923 by prof. BOUVIER, propolis is not a balsam but a resin made out of a multitude of different substances which may be isolated by solventation. The only interesting paper which exists about this subject is entitled "An Investigation of Canadian Propolis Grem", issued in the Canadian journal "The Canadian Chemistry" : under the signature of M.T.P. GLADSTONE-SHAW, expert-chemist, in 1925.

It is not possible to give details in this paper about this interesting study but it is quoted as reference.

Combs Contain Propolis

We have seen that propolis is a sort of cement used by bees to consolidate different parts of the hive ; what is even more surprising is the fact that they also use propolis to strengthen the natural combs adding it to the wax they secrete.

For a long time, people believed that these combs contained only pure wax. It was proved that it is not so : the reality is these combs also contain propolis and pollen. We emphasized this point of view in a paper which was presented by prof. VAYSSIERE from the Natural History Museum, on May 3, 1944.

Natural combs contain about 90—95% pure wax and 5—10% propolis which is added at the time the combs are built along with a considerable amount of pollen grains. These could even come from propolis which always contains pollen, no matter where its origin.

Harvesting

Propolis can be harvested by the beekeeper only by scraping the walls of the hive when changing hive equipment, or when replacing frames etc.

It is recommended that one should work at low temperatures because then, the propolis is brittle and it may be easily removed from the surface.

The amount harvested per hive depends on many conditions. The race of the bees is one of them : some bees collect much propolis, others little.

On average one may count on a crop of 100—300 g per hive.

The Use of Propolis

In general, the majority of the beekeepers who do not use propolis, do not pay any attention to it. Im my opinion they are not right, because they could obtain, firstly, by simply melting it a special wax, called by the late PERRET MAISONNEUVE, propolis wax. Thus, propolis wax is ductile, soft and may be used as a putty. Secondly, when dissolved in hot alcohol, propolis is an excellent varnish which will protect hives or prevent rusting of metal instruments used in laboratories.

Propolis was also used in former times in medicine. During the Boer war, at the end of the last century, dressings treated with propolis, protected wounds from gangrene : The physicians PARVEL and MEYER highly praised it in medical journals, after having experimented it in hospitals.

Such successful results were obtained during those times because this product is itself an antiseptic and it helps the formation of new flesh tissue.

The author has recently obtained a licence for using prepared propolis, in beauty creams and cosmetics.

Propolis as medicine

H. A. SAVINA and F. T. ROMANOV from the physiopathology section of the Veterinary Institute from Kazan, have prepared an ointment for use in the treatment of cuts, abscesses and wounds in animals. The method of preparing lies in mixing 100 g vaseline or animal fats, boiling, cooling to 50—60°, addition of 10 g of propolis, heating again at 70—80°, stirring for 8—10 minutes and covering the whole mixture for 8—10 minutes. It should then be filtered through a fine sieve. It is ready for use immediately after cooling.

This ointment has also been used for external ulcerations and on burns. Propolis is also used in dentistry in 2—4% semi-fluid solution.

One of my colleagues has marketed a plaster with propolis (poultice) which gives excellent results in cases of corns. This plaster also removes warts.

Propolis and Stradivarius

In an issue of the Portuguese apicultural journal "As Abelhas", there is an article in which Dr. Erich KNOPF says that he studied the characteristics of propolis from different sources and their use in the manufacture of varnishes for violins. This doctor is sure that the violins built by the famous Stradivarius have exceptional qualities due to the propolis harvested by the bees from the Cremona region.

Beekeepers' Dermatosis

There is still very little known about this allergy but it is very unpleasant for those who have it. It can be caused by food, perfumes, pollen, sun, in fact a multitude of things. The allergy has different symptoms which appear especially on the surface of the skin or on the mucuous membrane.

Some beekeepers become allergic to propolis. This is the beekeepers' dermatosis. It resembles eczema and it is manifest in the form of red spots and itching. The skin gets dry and it also becomes chapped.

The affected regions are the hands, the face, or the head if the beekeeper touches these parts.

As a remedy, oil is recommended and all sorts of other effiicient treatments. But the true healing was discovered by a beekeeper from Erevan : the chemist must prepare two parts of ammonia to 8 parts of glycerine in a flask. First, one must wash his hands with soap and water to remove the propolis. Then one has to rub his hands with the above mentioned solution.

People know that ammonia dissolves propolis. The skin goes yellow, but this can be rinsed off.

FROM THE HISTORY OF PROPOLIS

Z. A. MAKASHVILI
USSR

The healing characteristics of propolis have been well known from old times.

Propolis was used especially in antiquity, in Egypt. There, some thousand years B. C., propolis was very well known to the priests who had monopolized medicine, chemistry and the art of mummifying corpses.

The fact that propolis was also known to the old Greeks is demonstrated by the very Greek name of it.

There is an ancient reference which alludes to propolis. The famous Greek philosopher Aristotle, wishing to closely study bees' activity, built a transparent hive. But the bees did not want to reveal their "secrets" and they covered the inside transparent wall with a dark substance, probably propolis. (From the book by Hilda G. RANSOM *"The Sacred Bee")*.

The origin of propolis was the subject of a polemic between two Roman writers — Plinius and Dioscorides. The first, head the opinion that bees harvest propolis from the resin of willow buds, of the poplar, wild chestnut and other plants and the other writer assumed that bees harvest it from styrax. Data about propolis also appear subsequently, in the works of Gallen and Varro.

Abu Ali ibn Sina (Avicenna) distinguishes two kinds of wax in his well known work *"The Canon of Medical Science"* — the clean and the black wax. The clean wax is that which composes the combs walls where the bees rear the brood and store the honey, and the black wax is the filth of the hive.

It is clear enough that "the black wax" is propolis, which after Avicenna's testimony "has the characteristic of eliminating the spikes of the bolts and the stakes. It also rarefies, cleans and soaks". He also writes that "by its strong smell, the black wax makes you sneeze".

Along with other hive products, propolis is often mentioned in the Georgian medical treatises from the XIIth—XVth centuries. Here is an abstract from the Georgian book of medicine *"Carabadini"* (the author — Zaza Fanaskerteli — Titzishvili) in which the author proposes a device against mouth cavity swelling and against dental decays. It is prepared as follows : arsenic, red lentil, yarrow, wood germander, all crushed and passed through a sieve, and added to propolis. Then you add one spoon of olive oil and one spoon of honey.

These all are mixed and have to be put on the decayed tooth.

It is also interesting the fact that the Georgian lexicographer Sulhan-Saba Orbeliani (1658—1725) in the XVIIIth century gives an explanation in his encyclopedic dictionary, according to which "propolis is a substance similar to the wax from the bottom of the hive".

In folk Georgian medicine, they used ointments with propolis to cure some diseases.

There was the custom of placing a propolis cake on the belly button of the new born baby and also they rubbed children's toys with propolis.

The popularity of propolis can be accounted for not only by the ethical observations of "doctors" who were empiricists, but also by the fact that the beekeepers would gather it in great quantities, because Georgian bees use much propolis, especially on the walls of the hives, on the frames and on the inner cover.

A Georgian treatise of empirical medicine from the XVIIIth century, recommends the use of propolis in cases of haemoptysis. "One has to take propolis grains having the size of a pin's head, for a couple of days — 3 pieces, in the morning and in the afternoon".

In the last few years, a commission for the study of Georgian orthodox medicine has established that the therapeutic values of propolis are still used at the present time in orthodox medicine. This knowledge was originally propagated by word of mouth from generation to generation and it was often kept as a secret.

Here are some folk recipes :

A heated propolis cake is applied on the sick place in the case of pains caused by cold. In the case of rheumatic pains to the extremities, a heated propolis cake is applied on the painful region. The pro-

polis cake must be wrapped in a warm cloth and left in place for the whole night.

In cases of furunculosis, a thin heated propolis cake is applied on the furuncle, which begins eliminating the pus in a short time.

In order to get rid of corns, one first deeps his feet in warm water, then puts a thin layer of heated propolis on the corn, and then bandages the foot.

DEFENDING THE BEE TOWN

A. B. NIKOLAEV
USSR

The interest in propolis stems from very ancient times ; its very denomination (Pro — "in front of" or "before" and Polis — "town") shows that for the hive — "the bee town" — propolis is a defence wall. Indeed, when unexpected guests come to the hives — insects, snails, lizards, mice, frogs — to rob honey, then the bees, after having killed them, cover them with propolis. The corpses so covered are transformed into mummies and never decay. In order to prevent the access of these visitors, the bees use propolis for repairing and building.

They stop all the gaps and holes with it, make the entrance of the hive smaller, level the different ruggednesses from the inside of the hive. That is why propolis is also often called bees' glue. The bees also smear the cell walls of the comb with propolis, before the queen lays eggs in them which ensures a good disinfection of the hive. By covering the inside walls of the hive with propolis, the bees are thus protected from cold in winter and from excessive heat in summer (in the above mentioned case the propolis is used as a thermic insulator).

Propolis is one of the most valuable of apicultural products, but its chemical composition is still insufficiently known. Propolis is a resin, being dark green or brown in colour, with a pleasant flavour of poplar buds, honey, wax and vanilla but it can also have a bitter taste. When burnt, it exhales a smell of aromatic resins of great value. Propolis contains about 55% resins and balsams, 30% waxes, 10% etheric oils, and 5% pollen. These components are rich in vitamins and minor elements.

The resins and balsams from propolis contain cinnamic alcohol, cinnamic acid, and tannant substances.

In propolis, there has been discovered a salivary gland secretion of the bees as well as other accidental components.

From older times, they thought that bees collected propolis from the buds and branches of poplar and willow as well as from the buds of birch, alder, horse chestnut, elm, from some herbs, and occasionally from the buds of pine and spruce fir. This hypothesis is also supported

by contemporary beekeepers. But there are also other opinions that bees collect the resin secretions of the buds and small branches, only when the pollen of flowers is insufficient and the bulk of propolis is processed by bees as a by-product during the digestion of pollen. It is known fact that the husks of pollen contain oily and balsam substances, resins included.

These substances prevent the content of the pollen grain from structural alteration under humid and in other unfavourable conditions. When the bees prepare food from pollen for their larvae, they put aside the undigested part, that is, the balsam resinuous substances which are deposited in the form of propolis drops. These are used afterwards for the needs of the hive. It is probable that bees collect propolis using both methods as stated above. It is enriched by the addition of a digestive ferment, and then processed by a lactic fermentation in their digestive tracts.

In order to obtain the cleanest and best propolis, without any alien mechanical additive, it has to be collected in summer when the bees finish their main honey flow. In one season, one may obtain 100—150 g propolis from each hive, and for the experienced beekeepers, who can stimulate the bees in this activity, they may succeed in obtaining even 400 g of propolis per hive.

The economic value of propolis is very important. On the technical side propolis is the raw material for obtaining some fine varnishes for furniture and stringed musical instruments. It is a known fact that Stradivarius used to varnish his violins with propolis. The resistance of the varnish that has the addition of propolis is so great, that the varnished surface is not destroyed even when boiling water is poured on it.

The therapeutic characteristics of propolis have been well known for a very long time. This is explained by its very pronounced microbial characteristics.

Propolis was used effectively on wounds by doctors during the Anglo-Boer war, and during the World War II it was also used in hospitals.

In folk medicine, the use of propolis is widely known especially for the treatment of corns. People inhale propolis in cases of affections of the respiratory tracts and of the lungs. It is also efficient for burns and angina.

From 1969, orthodox medicine in U.S.S.R. accepted the use of "Propolis-30%" (30% alcoholic solution of propolis). It is produced by the pharmaceutical products plant in Tallin.

"Propolis 30" is recommended as an external remedy for the treatment of chronic eczema, of dermatoses, burns etc. Propolis products, as therapeutical agents, can be applied according to a physician's recommendation.

ANALYSIS AND CONSIDERATIONS ON THEORIES CONCERNING PRODUCTION OF PROPOLIS

J. ČIŽMÁRIK, M. MAČIČKA, I. MATEL
CZECHOSLOVAKIA

In the early days of beekeeping practice, propolis was usually considered to be beeswax. Later on, when the natural history of the honey bee has been investigated, it was found that propolis was a building and protective material with which bees plug the cracks in the hive, and that it also has other characteristics than beeswax. At that time the question arose : which is the composition of propolis ?

The first theories about the composition and origin of propolis were very primitive but as new knowledge about the life of the bees was acquired the previous has had to be rejected. At present there are only suppositions along the road leading to the theories about the composition of this bee product.

Nowadays, two theories have been advanced concerning the composition and origin of propolis.

According to the first, bees collect propolis from the resins and secretions of buds and from the bark of resinous and deciduous trees around the apiary. The supporters of this theory hold that bees collect propolis in the following manner : first, they remove by means of their mandibles a piece of resin or secretion, which they then proceed to process by the same mandibles. Next, propolis is processed by the forelegs, then transferred to the midleg, and finally, into the basket on one of the hind legs. The bee thus forms pellets just as with pollen. With this load of resin, the bee flies back to the hive where other bees take the pellet and use it where necessary. Major supporters of this theory include RÖSCH, EVENIUS, BERLEPSCH, CIESELSKI, and others. Because Dr. RÖSCH was the first to suggest this theory it was acknowledged as RÖSCH's theory about the composition and origin of propolis.

In 1907, Dr. KÜSTENMACHER advanced the theory that propolis was derived from pollen grains. According to him, worker bees ingurgitate pollen and accumulate it in one of the section of the intestine *Chylus magen* — which he calls the "pollen stomach". The process of the formation of propolis begins by the absorption of a great quantity of water in pollen. The pollen grains swell as they absorb a quantity of water 5 times greater than their weight, breaking after. Plasma leaks from them, which bees use as food for the young bees which feed brood. From the husks of the pollen grains, a balsam is produced which bees eliminate as 2—3 mm drops. According to Dr. KÜSTENMACHER this balsam is the basis and essence of propolis.

But not all pollen grains are of the same quality and some of them do not break even when swollen ; hence they are a worthless stuff as food and bees try to eliminate them. Because of its lower specific density, this worthless stuff reaches the inferior section of the intestine where it combines with the balsam. By wagging movements of the "pollen stomach" the worker bees eliminate and store on the walls of

the hive or in cracks the mass of balsam and the undigested pollen grains. There, a yellow-reddish mass is formed which solidifies. Before having solidified completely, bees add to it — inside the hive — dust and mechanical impurities which make the stuff have a certain consistence allowing it to be carried from place to place.

Consequently, according to Dr. KÜSTENMACHER, propolis is produced from the outer of pollen grains, which bees blend with wax, with other *additional* materials — especially of pollen type, and with impurities in variable quantities. "In spite of close watching, I have seen no bee collecting resin from buds and therefore such an idea is beyond my belief" says the German researcher in conclusion of the account on his investigations.

It is obvious, the theory of Dr. KÜSTENMACHER concerning the production and derivation of propolis is fundamentally different from that of Dr. RÖSCH.

Actually, all the other concepts which have been advanced so far about the production and derivation of propolis are in principle similar in detail to the first or second theory. But which is the correct one?

The fundamental principles of apicultural science have up to now supported or invalidated parts and details of both theories; consequently, it is necessary to consider and examine them objectively. According to recent knowledge, and on the basis of the results obtained, a new more objective theory about the elaboration and production of propolis has emerged.

Let us approach at least a few aspects of the two theories. As a basis for examination and consideration we could refer to the partial results obtained from the study of the chemical composition of propolis.

If propolis were formed on the basis of pollen as assessed by Dr. KÜSTENMACHER, it should include more nitrogen and lipid substances and sugars such as are found in pollen in relatively great amounts. But the results of the chemical analysis of propolis obtained so far do not confirm this. Neither were found in propolis, other substances which exist in pollen even in small quantities. Also invalidating this theory are physiological, morphological, biological and anatomical evidence which have been singled out by HAYDAK, EVENIUS, and RÖSCH — which we do not specifically mention in this paper.

The investigation of propolis made of late have produced much objective evidence in support of Dr. RÖSCH's theory. The study of the chemical composition of propolis showed that this theory is more thoroughly grounded and much closer to reality.

Most significant is that according to this theory the substances which have been ascertained to exist in propolis are surely found in one or several plant species which bees visit, whose secretions could be potential sources of propolis.

But of paramount importance is the fact that in the present stage of knowledge the honey bee is ascertained to take an active part in producing propolis; by means of its glandular secretions, and from vegetal sources the bee produces propolis as it is found by the beekeeper, in the hive. This assertion attests to the fact that most flavonoid

substances identified in and isolated from propolis were found as free substances as aglucons not glycosides, just as in vegetal matter. Hence, the bee must have a substance of decomposing glycosides in their major components which it subsequently processes to obtain propolis and food. It is the experts in biochemistry of the honey bee who will identify this substance and its chemical composition. A solution in this respect would be an important step forward in elucidating the composition and origin of propolis.

As the results above show there is still much to be done to establish the composition of propolis, a debated problem for which solution apicultural science is still searching. As one of the possible methods to be used in this respect we suggest analysis with isotopes, a method which imposes no difficulty — either systematically or materially.

II. CHEMICAL COMPOSITION OF PROPOLIS

CHEMICAL COMPOSITION OF PROPOLIS, ITS ORIGIN AND STANDARDIZATION

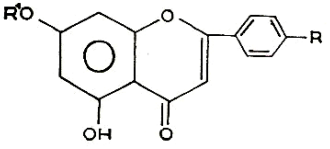
S. A. POPRAVKO
USSR

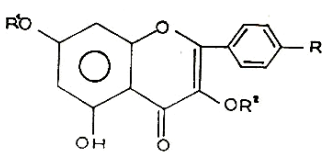
The future success of propolis in daily practice depends upon a knowledge of its chemical composition, the biological activity of its different components and being able to standardise it as a product.

At the Institute for Chemistry of Natural Compounds under the co-ordination of the U.S.S.R. Academy of Sciences, a thorough study for these problems was conducted; the results are presented in this report.

Chemical Composition

In the last few years, considerable progress has been achieved in obtaining a knowledge of the chemical composition of propolis. Up to the present, we know the chemical structure of 18 components of propolis and 11 of them have been isolated and identified in our laboratory. We give below these components which make up at least 1/3 of this substance, dissolved in alcohol. The main components of propolis are flavones (1—4), flavonoles (5—10) and flavonones (11—13). There was also identified a terpene from the group of caryophyllene — α-acetoxy-betulenol (15) and an aromatic aldehyde-isovanillin (16).



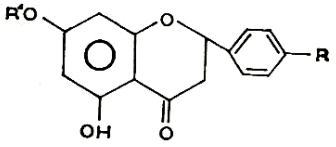


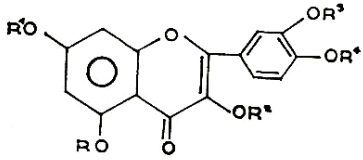
Flavones

(1) R^1 = H,R = H (chrysine)
(2) R^2 = Me,R = H (tektochrysine)
(3) R^1 = H,R = OMe
(4) R^1 = Me,R = OMe

Flavonones

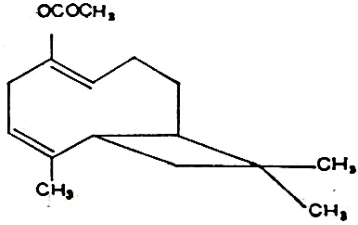
(5) R = H,R^1 = R^2 = H (galangyne)
(6) R^1 = Me,R^1 = R^2 = H (isalpinine)
(7) R^1 = Me,R^2 = H,R = OMe
(8) R^1 = H,R^2 = Me,R = OMe
(9) R^1 = Me,R^2 = H,R = OH (ramnocitryne)
(10) R^1 = H,R^2 = H,R = OMe (kempheryd)

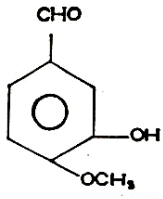


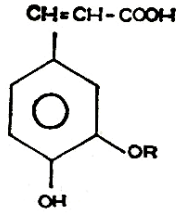


Flavonoles

(11) R^1 = P = H (pinocembryne)
(12) R^1 = Me,R = H (pinostrobyne)
(13) R^1 = Me,R = OMe







Quercetin Derivatives

(14) R^1—R^5 = H or Me
(15) α-acetoxi-betulenol ; (16) isovanillin ; (17) R = H
(18) R = Me

The Origin of Propolis

The majority of these components have been isolated so far from different plants and some of them (8) and (13), for instance, are described for the first time.

In propolis, as the Czech researchers ČIŽMÁRIK and MATEL found out there are also aromatic, unsaturated acids, caffeic and ferric acids (17, 18), which are characterized by their biological activity.

The isolated components (3—4, 7—10, 12—16) have a common feature, namely, that they can be found in most types of propolis which are obtained in the main apicultural zones of USSR.

The Origin of Propolis

By being able to establish the structure of most propolis components and the development of some efficient chromatographic methods for their analysis gave us the opportunity to typify the chemical com-

position of propolis samples collected from the main apicultural regions of the USSR.

The chromatographic analysis was performed on plates with a thin layer of Silufol, with ethylbenzen acetate (1 : 9) and normal ethylheptan acetate (2 : 3) as solvents. The corresponding spots of the component substances were revealed by spraying the plates with sulphuric acid, with 0.5% benzaldehyde and then, by exposing the plates to 80° for 1—2 minutes.

These tests proved that on every 10 propolis samples collected on the European teritory of the U.S.S.R., 8 were more or less identical in their chemical composition.

We analysed these samples and were able to show that in samples of the same type, the components 3—4, 7—10, 12—14 and 15 are always present.

The above-mentioned composition of propolis samples from a large geographic territory, clearly indicates the common source of raw material used by bees for preparing propolis. The identification of this source on a chemical taxonomy basis, proved by the chromatographic analysis of pollen fractions, buds of different wood essences, some plant secretions, and resins show that propolis is a mixture of substances which are released by birch buds during this dormant period. From the alcohol extracts of birch buds *(Betula verrucosa)* we isolated and identified the same components almost in the same concentration as in propolis (3—4, 7—10, 13—16). Referring to other components still unidentified in the type of propolis indicated above, and from birch tree exsudates, they are identical from a chromatographic point of view.

According to these data, the commonest type of propolis with the above compounds is birch propolis. Another type of propolis widely found in the European territory of U.S.S.R., is poplar propolis. Its chemical composition corresponds exactly to that of poplar bud secretions (Populus nigra). This type matches especially the 1, 2, 5, 6 and 11 components.

The resemblance between the chemical composition of the poplar bud secretions and that of the propolis harvested in France has also been shown by LAVIE et al. for a long time.

Our chromatography tests have confirmed this resemblance, on many samples of propolis, especially those harvested from poplar zones in our country.

We discovered another plant source of propolis. The chromatography analysis of the pellets on the bees' legs, harvested from a plant unidentified so far, has shown that two compounds with Rf 0.40 and Rf 0.15 are present in the system of ethil acetate normal heptane (2 : 3). Both of them get dark red after having sprayed the spots with sulphuric acid. The substance with Rf 0.40 which is to be found in the birch type propolis was isolated in pure form, both from propolis and from the pellets on the bees' legs. The physico-chemical analysis indicated that the substance is an aromatic compound with molecular weight 284 ; it includes the phenol and methoxi groups and also an unconjugated carbonyl group.

The indicated sources for the component substances of propolis are accessible to bees in our territory from the month of June till the end of the season. Concerning pollen as a possible propolis source, our researches have shown that this does not hold true, because the pollen spectrum does not contain the permanent characteristic components of propolis. Thus, KÜSTENMACHER's theory cannot be based on scientific fact.

Minute research of the chemical composition of propolis has confirmed not only its origin but it has also shown that many plant species contain this important product for sustaining the life of a bee colony.

The Standardization of Propolis

The discovery of permanent components in propolis and of its main types facilitates the solving of the standardization problem of propolis.

It has been found that in U.S.S.R. there are, according to the chromatography analysis of about 90 samples from different zones apart from the Far East, Central Asia and Caucasus, 4 types of propolis :

(1) birch type 65%
(2) poplar type 15%
(3) birch-poplar type 15%
(4) other types 5%

Because birch propolis and the poplar type cover practically most of the samples and include a great number of chelate compounds structurally related to the phenolic ones, we were able to establish a rapid method of estimating the origin of small quantities of the substance (8—10 mg). Using larger quantities (0.1—0.5 g) we could find the ratio of the products main components and thus we could characterize each of them.

The basis of rapid determination methods is the colour reactions of a 0.1% alcohol solution of propolis, with complex reagents such as water solutions of iron chloride, copper acetate, lead acetate etc. from which one can also establish the presence and quantity of flavonoids (components of the types 1—10) and also the reaction with 20% sodium hydroxide. These isomerize the flavonoles (components of the types 11—13) and are always present in propolis, in coloured chalkones.

In order to characterize the different propolis some researchers use their adsorbtion on alum earth (to the IInd degree of activity). They also use the ability of full solubility aluminium earth in chloroform-acetone (2 : 1), which finally permits the diferentiated quantitative determination of the amorphous fraction of propolis and of the biologic ally active one.

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SOME CHEMICAL AND PHYSICAL DATA ON ROMANIAN PROPOLIS

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Propolis, a natural bee product, has a complex chemical composition which has not been cleared up completely as yet.

As this is gathered by bees from very different plants its chemical composition varies very much. This determined some researchers to make chemical analysis of propolis coming from different areas.

This hive product proved to be particularly important by its biological activities especially by its antimicrobial action and by its use for some therapeutic purposes in human medicine and veterinary science. That is why the study of its chemical composition and the determination of the components responsible for its biological activity is of much interest and at the present time there are numerous interesting works dealing with chemical composition of this hive product (1, 2, 4, 7, 8, 10, 11).

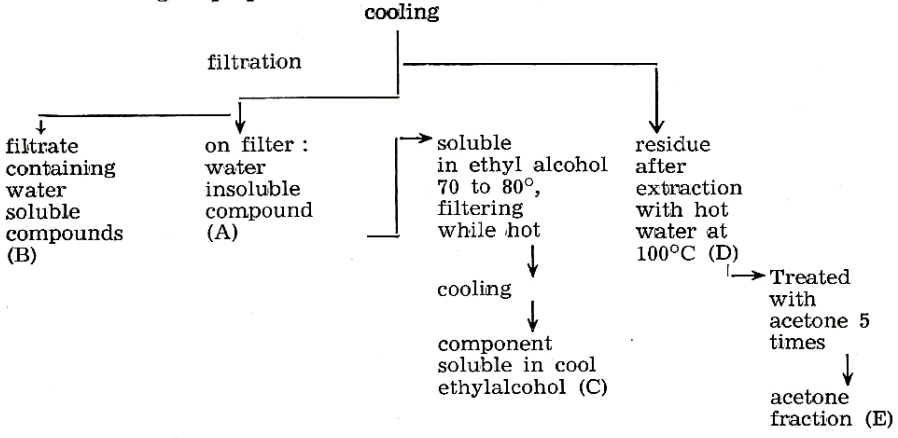
The object of our work is to study the chemical composition of some propolis harvested by us in order to determine its components of protein nature, derivates of nucleic acids and organic solvent soluble compounds. The methods applied were chromatography, electrophoresis, UV absorption and some chemical reactions.

Material and method

In the first set of experiments we tried to analyse the fraction obtained by hot extraction with water, to determine the presence of flavonoids, the UV absorption of watery extract and the residue left after treating propolis with water.

To this end 15 g of propolis (diagram 1) are treated with 80 ml double-distilled water in a porcelain dish. The mixture is gradually heated and allowed to boil for 3 minutes after which it is cooled in the

Diagram 1
TECHNIQUES USED TO SEPARATE DIFFERENT FRACTIONS FROM PROPOLIS



refrigerator. Hot extraction and cooling are repeated 3 times. After cooling, water is filtered and on the filter we obtain component A insoluble in cool water. It is put in the exsiccator and afterwards it is weighed to calculate the percentage of content.

Samples of fraction A were dissolved in a mixture of chloroform-methanol (80:20) and analysed by circular paper and thin layer chromatography.

The filtrate obtained by filtration of cooled water is fraction B and contains compounds extracted by hot water which remain soluble in water after its cooling.

This fraction (B) was analysed to determine : the reactions showing the presence of flavonoids by using Na OH_2N or concentrated H_2SO_4 (11) and UV absorption, the samples being read between 370 mμ and 230 mμ.

The residue left after hot treatment of propolis with water is put in the exsiccator, crumbled as it dries and weighed until obtaining approximate constant weight necessary to calculate the percentage of content.

This fraction marked D is the insoluble residue left after treating propolis with hot water 3 times.

5 g of residue D are extracted in 5 successive stages with 150 ml acetone at room temperature in the dark. Acetonic extracts are reunited and they represent acetonic fraction E which was analysed by disc paper and thin layer chromatography.

The water insoluble fraction A is dissolved in 80 ml of ethyl alcohol and heated up to 70—80°C. It is filtered at 70° and the filtrate is cooled in the refrigerator.

The component insoluble in cool ethyl alcohol is separated by cold filtration after which it is put in the exsiccator where it is kept until obtaining the constant weight and the percentage of content is calculated. This fraction marked by C contains compounds soluble in ethyl-alcohol 70—80° and insoluble in cool ethyl alcohol.

Samples of fraction C were analysed by circular paper and thin layer chromatography.

As to the methods applied, disc paper chromatography was performed according to indications given by HORACHEK and TCHERNIKOVA (5). For the 3 migrations there were used the mixture chloroform-methanol 80:20 (1st migration), acetone (2nd migration) and methanol (3rd migration).

The paper Whatman No 1 discs on which there were drawn 3 circles for the 3 migrations, was washed 2 times with the mixture chloroform-methanol 80:20. Development was performed by using osmic acid.

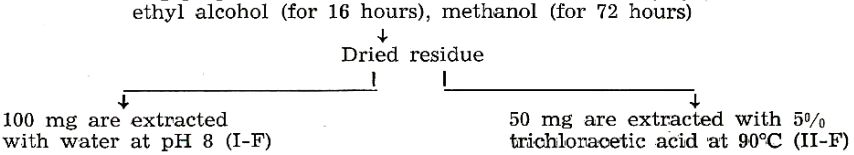
Thin layer chromatography was made on plates 20×20 cm covered with silicagel M and activated for 1 hour at 110°. The system used for migration was the mixture cyclohexane-ethyl-acetate-chloroform (40:10:1). Development was made by molybdenic acid 10% in methanol heated for 5 to 10 minutes at 120°.

In the 2nd set of experiments we were interested in determining the presence in propolis of compounds of protein nature and derivates of nucleic acids.

2 g of propolis are treated 5 times with 50 ml acetone for 20 days at room temperature in the dark. Acetonic extracts are removed, then the propolis residue is treated with ethyl alcohol for 16 hours and then with methanol for 72 hours. Then it is placed on filter paper, washed several times with methanol and put in the exsiccator until its drying. This fraction of propolis from which compounds soluble in the above mentioned organic solvents were removed occurs as a white-greyish product marked F, which was analysed for determining the presence of compounds of protein nature and derivatives of nucleic acids.

Diagram 2

OBTAINING THE PROPOLIS FRACTION FROM WHICH COMPOUNDS SOLUBLE IN ORGANIC SOLVENTS WERE REMOVED



To this end 100 mg of fraction F were extracted with 3 ml double distilled water at pH 8 for 1 hour. With the filtrate thus obtained (I-F) there were performed : a Mejbaum reaction with orcinol (6), a Dische reaction with diphenylamine (2), the UV absorption between 370 mμ and 230 mμ and the polyacrylamide gel electrophoresis.

The samples for Mejbaum reaction were prepared with 0.6 ml filtrate and 0.2 ml Mejbaum reagent and for Dische reaction with 0.6 filtrate and 0.6 ml Dische reagent.

Polyacrylamide gel electrophoresis was performed with 0.2 ml tube filtrate, boric trisacid — pH 8.2, migration time — $3^1/_2$ hours, development — with Amidoschwartz.

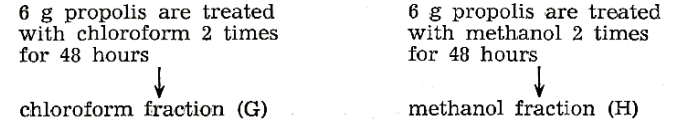
Separately, 50 mg of fraction F extracted with 2 ml of 5% trichloracetic acid for 15 minutes at 90° according to SCHNEIDER (9) were centrifuged after which with the supernatant II F there were made the Mejbaum and Dische reactions and the UV absorption.

The 3rd set of experiments were conducted to determine the presence in propolis of compounds soluble in organic solvents namely in chloroform and methanol.

To this end 6 g of propolis (diagram 3) are treated 2 times for 48 hours with chloroform at room temperature in the dark. The reunited fractions constitute the chloroformic fraction G.

Diagram 3

OBTAINING 2 PROPOLIS FRACTIONS : THE CHLOROFORM AND METHANOL FRACTIONS



ANALYSIS OF FRACTIONS EXTRACTED

Fractions	Content %	ction for ivonoids	Reaction with orcinol	Reaction with diphenylamine
1. Water insoluble fraction (A)	10%			
2. Water soluble fraction (B)		NaOH 2N++ H_2SO_4 Concentr. ++		
3. Ethylalcohol soluble fraction at 70—80° ; insoluble in cool ethylalcohol (C)	3.7%			
4. Propolis residue left after extraction with water at 100° (D)	78%			
5. Acetone fraction of residue D from propolis (E)				
6. Fraction extracted with water at pH 8 from propolis treated with acetone-ethylalcohol-methanol (I-F)			+	—
7. Fraction extracted with 5% trichloracetic acid at 90° from propolis treated with acetone, ethylalcohol and methanol (II F)			++	—
8. Chloroform fraction (G)				
9. Methanol fraction (H)				

Another propolis sample was used to perform the methanol extraction, thus obtaining methanolic fraction H.

These 2 fractions were studied by disc paper and thin layer chromatography.

Results and discussions

By applying the following methods of analysis : disc paper and thin layer chromatography, polyacrylamide gel electrophoresis, UV absorption spectrum and chemical Mejbaum and Dische reactions, we succeeded in showing the presence of different compounds that differ in chemical nature and physico-chemical properties. By treating propolis with dif-

Table 1

FROM PROPOLIS BY DIFFERENT SOLVENTS

UV absorption	Polyacrylamide gel electrophoresis	Circular paper chromatography	Thin layer chromatography
		Soluble compounds in : chloroform-methanol, acetone, methanol	9 spots
maximum 30 mμ			
		Soluble compounds in : mixture chloroform-methanol, acetone	3 spots
		Methanol soluble compounds	9 spots
No maximum between 370 and 230 mμ	8.9 fractions		
No maximum between 370 and 230 mμ			
		Compounds soluble in : mixture chloroform-methanol, acetone, methanol	11 spots
		Compounds soluble in methanol	11 spots

ferent solvents such as chloroform or methanol one could point out the presence of various groups of compounds that differed in point of their solubility in organic solvents and the number of compounds they contain.

In the first place one could ascertain that chloroform propolis extract (G) analysed by disc paper chromatography contained 3 groups of compounds that differ in their solubility, namely : 1) compounds soluble in the mixture chloroform-methanol and insoluble in acetone and methanol (which migrate to circle 1); 2) compounds soluble in acetone and insoluble in methanol (migrating to circle 2) ; 3) methanol soluble compounds (migrating in circle 3) (fig. 1, G). The thin layer plate chromatography of chloroform extract shows the presence of 9 spots (fig. 2 G).

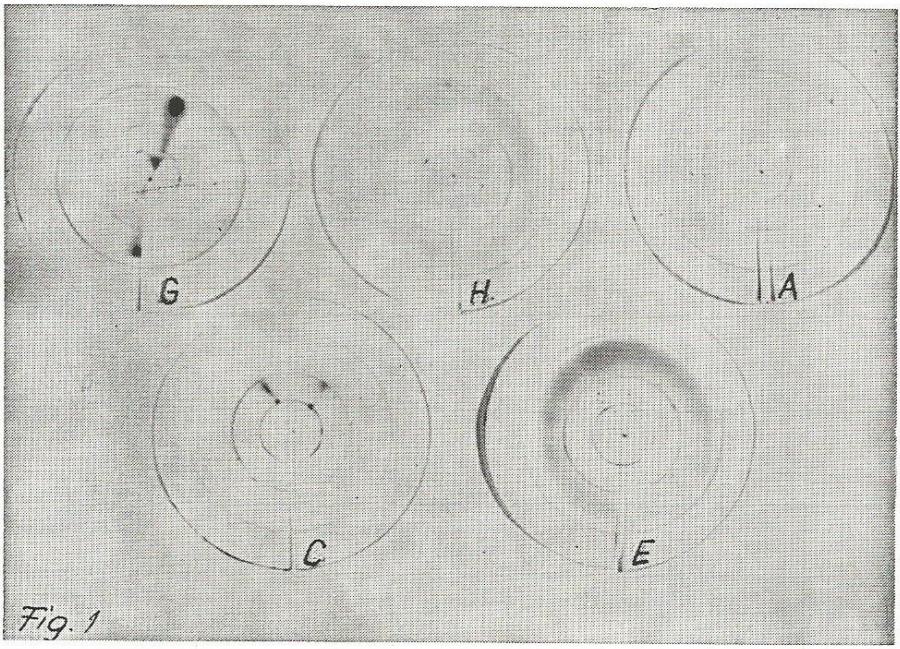


Fig. 1 — Circular paper chromatography of fractions G, H, A, C, E extracted from propolis

Unlike the chloroform extract the methanol one shows by circular paper chromatography a single group of compounds that migrate to circle 3, hence methanol soluble (fig. 1, H) and which by thin layer chromatography show the presence of 9 compounds (fig. 2, H). It can be seen that the thin layer chromatography of the 2 chloroform and methanol extracts differ.

Fraction A, as already mentioned, is obtained by hot water extraction and then separated from water by cooling it. This fraction accounts for 10% of the total content of propolis. The analysis of fraction by the two chromatographic methods shows that it contains also compounds soluble in chloroform-methanol, in acetone and in methanol, hence compounds migrating to circles 1, 2 and 3 (fig. 1, A), and thin layer chromatography shows 9 spots (fig. 2, A).

The analysis of fraction B soluble in cool water showed the presence of flavonoids, the reaction being positive with both NaOH 2N and concentrated H_2SO_4 (table 1).

Fraction A is complex. Generally it contains beeswax as well as other components wich are carried away together with separated beeswax after cooling of water.

Fraction C soluble in hot but insoluble in cool ethyl alcohol accounts for 3.7% of total content of propolis. The paper chromatography points out 2 groups of compounds : some soluble in the mixture chloro-

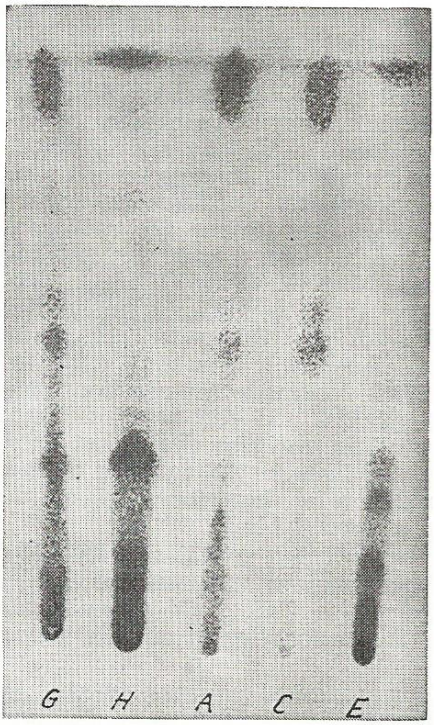


Fig. 2 — Thin layer chromatography of fractions G, H, A, C, E extracted from propolis

Fig. 3 — Polyacrylamide gel electrophoresis of fraction I-F extracted from popolis

form-methanol and insoluble in acetone or methanol and some soluble in acetone (fig. 1 C).

The thin layer chromatography of fraction C shows 3 spots (fig. 2 C).

Propolis residue left after treating propolis 3 times with hot water and then dried accounts for 78% of total propolis (fraction D).

Fraction D was repeatedly extracted with acetone. The analysis of reunited acetone extracts (fraction E) and their paper chromatography reveal a single group of compounds that migrate to circle 3, hence methanol soluble compounds (fig. 1 E). This does not exclude the possibility of their solubility in the first solvents used for paper chromatography too. The thin layer chromatography of fraction E showed that it contained 9 compounds because the chromatograms once developed showed 9 spots (fig. 2 E). One can see that fraction E contains very few compounds of those removed by hot extraction which are found in fractions A and C (fig. 1 A, C, E).

The 2nd set of experiments aimed at determining the chemical composition of propolis, particularly the compounds of protein nature and the derivates of nucleic acids.

Consequently it was necessary to obtain a fraction of propolis free from any compounds capable of affecting electrophoresis or reactions with orcinol and diphenylamine. To this end the compounds soluble in organic solvents were removed from propolis. They were chosen depending upon the possibility of their extraction by as many and different means as possible. Similarly only those miscible with organic solvents were chosen. The polyacrylamide gel electrophoresis of aqueous extract (fraction I F) of propolis treated previously with acetone and methanol revealed a number of 8—9 fractions which were colored by Amidoschwartz (fig. 3). It should be mentioned that A. DEREVICI (1), who studied the chemical composition of the same propolis, namely the amino-acids, by making the acid hydrolysis and the paper chromatography, showed the presence of 8 amino-acids. As to the amount of the derivatives of nucleic acids, RNA and DNA, we made the reaction with orcinol to determine the presence of ribosis, and the reaction with diphenylamine to determine deoxiribosis. The analyses show that only the reaction for determining ribosis was positive in the aqueous or the trichloracetic acid extract whereas the reaction for deoxiribosis was negative under the conditions of our working method (table 1).

By plotting the UV absorption curves between 370 mμ and 230 mμ for the same fractions (I-F and II-F) no characteristic maximum could be obtained of the azotate bases of nucleic acids. This could be explained by presence of different compounds in the extract which did not allow to obtain a maximum of 260 mμ.

It should be noted that the UV absorption of aqueous fraction B resulted in a maximum absorption at 300 mμ.

In conclusion, our analyses show that propolis contains in the first place groups of compounds soluble in organic solvents which differ in their solubility and content of compounds separated by thin layer chromatography. These compounds showed colour reactions with osmic and phosphomolybdenic acids.

The polyacrylamide gel electrophoresis of a water extract revealed the presence of 8 to 9 fractions that can be colored by Amidoschwartz whereas the analysis aiming at determining the derivatives of nucleic acids showed but the presence of a positive reaction for ribosis.

Similarly there was pointed out the presence of flavonoids and a maximum absorption at 300 mμ.

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CHEMICAL COMPOSITION AND BIOLOGIC ACTIVITY OF PROPOLIS

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The ever growing interest in propolis during the last few years came about because of its many-sided biologic activity. In this connection, we began studying the biologic activity of the main propolis type collected by bees in the European territory of U.S.S.R.

This type of propolis is characterized by the presence of the following compounds : α-acetoxybetulenol, 5-hydroxy-4′, 7-dimetoxy-flavone, 3,5-dihidroxy-4′, 7-dimetoxyflavone, 5,7-dihidroxy-3,4 dimetoxyflavone, 5-hidroxy-4′, 7-dimetoxyflavone, 5,7dihidroxy-4′ metoxy-flavone, 3,5,4′-trihidroxy-7-metoxyflavone, 4′,3,5,7-tetrahidroxy-3′-metoxyflavone, 3,5,7-trihidroxy-4′-metoxy-flavone and it is known as *birch propolis*.

By the chromatography of the alcohol extract of this propolis on silicated column with IInd degree activity in oil ether-benzene and benzene-acetone — we isolated 20 fractions. Each of them had to undergo a biological examination in order to see the antimicrobial activity on the following cultures : *Staphylococcus aureus, Sarcina lutea, Streptococcus faecalis, Candida albicans, Saccharomyces cereus, Bacillus mycoides, Bacillus subtilis* and *Mycobacterium phlei*.

This study was performed according to the method for serial cultures, by using alcohol or dimetylformamide. It was shown that the main antimicrobial activity was concentrated in the fractions 7, 8 and 9.

In the case of *Staphylococcus aureus*, the activity is 60—70 mg/ml. The chemical composition of these fractions has shown that among their compounds, the following should be mentioned : 5-hidroxy-4′,7-dimetoxy-flavone, 3,5-dihidroxy-4′,7-dimetoxyflavone, 5,7-dihidroxy-2,4-dimetoxy-flavone and essential compounds, with 284 molecular weight. Some of these compounds are derivatives of 3-hidroxyflavones and are characterized by an important biological activity, however not greater than that of the initial fractions, which indicate either the presence to a small extent of more active compounds in these fractions or a synergetic action of these compounds.

CONTRIBUTIONS TO INVESTIGATION OF COMPOSITION OF PROPOLIS

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CZECHOSLOVAKIA

At the Laboratory of cosmetics at the Research Institute for fats industry we have also made investigations into the components and qualities of propolis. As we consider that interesting conclusions have been reached, we shall briefly describe our tests.

First, we examined the characteristics of the extracts obtained by various solvents from 11 samples of propolis. The tests were made within the ultraviolet range of the spectrum in order to find out whether propolis extracts can be used as filters against solar radiation. The following conclusions have been reached :

a. The best results were obtained with 1 : 10 extracts in ethyl alcohol, obtained in 24 hours ;

b. All extracts within the 200—370 range exhibited specific peaks for wave lengths of 235, 285, and 314 mμ, and certain differences according to quantity ;

c. The activity of propolis extracts does not depend on their colour.

This is especially the characteristic of derivates of cinnamic acid and of a number of substances based on gallic acid.

Subsequently we separated these active principles after which we obtained a specific crystalline mixture which we have thoroughly investigated.

Material and Method

We removed the beeswax from propolis by heating it twice in 350 ml of water. The resulting substance was distilled in water vapour, a distillate of 3 : 1 being obtained. After filtration of the distillate we extracted the ethyl ether three times. Each time the ether layer was removed. From the combined ether extracts, approximately 50 ml of distillate were obtained. For 20—24 hours and occasionally over an even longer period typical acicular crystals covered by an oily pellicle would separate from this solution. The crystals were sucked, washed with 1 : 1 ethyl ether-petroleum ether mixture and were recrystallized from ether. The melting point of this substance was 115—118°C. By microscopic examination, together with crystals also small oil drops were identified. The crystalline substance obtained after four distillations was then subject to fractional sublimation. Three fractions were obtained. The first fraction included mostly oil substances, the second — crystals, and the third — some impurities. The second fraction was recrystallized in ether, the melting point of the substance obtained being 122°C.

The substance was analysed by spectroscopy in infra red spectrum and by nuclear magnetic resonance test, and it was identified as benzoic acid.

We further checked the substance by thin layer chromatography, in three various systems :

a) In the acetone-petroleum ether system, 1 : 3, on Silufol according to Lyman. The R F coefficient is the standard for the benzoic acid : 0.35;

b) In the ethanol-water-ammonium (25%), system, 25 : 3 : 4, on Silufol, according to BRAUN and GREENEN. The R.F. coefficient is the standard for the benzoic acid.

c) In the ethyl ether-petroleum ether system, 3 : 7. The R F coefficient is the standard for the benzoic acid (0.7).

Next, the substance was checked on chromatography in dextran gel ; the benzoic acid was identified by comparing volumes with the standard. In fraction No. 2 we also identified an unknown substance. This substance was also identified by thin layer chromatography, in all the systems mentioned above. It is an intensively fragrant substance, which at 254 mμ releases an intensive clear blue fluorescence. According to the knowledge available, we concluded that it is an aromatic substance, with one nucleus and a greater molecular weight that the benzoic acid. The substance was identified, in various quantities, in the three fractions obtained by fractional sublimation. In the first and third fractions, together with the two substances identified — the benzoic acid and the fluorescent substance — also many other substances exist. For the identification of the substance accompanying the benzoic acid we shall make further investigations in order to find out whether this substance which exists in propolis is an already known or an unknown substance.

From our experiments benzoic acid was confirmed to exist in propolis. Of 150 g of propolis we obtained 1—2 g of crystalline mixture of which approximately 50% was benzoic acid. The quantity of crystalline mixture obtained, depends on the amount of beeswax-resins found in the various propolis samples.

The existence of benzoic acid in propolis is due to its presence in numerous plants — clover, cowberry, poplar buds etc. from which bees collect it together with other substances.

The effect of the benzoic acid upon micro-organisms is well known, and consequently it has its share — alongside with other substances — in the bacteriostatic and bactericidal effects of propolis.

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STUDY OF THE CHEMICAL STRUCTURE OF PROPOLIS ISOLATION AND IDENTIFICATION OF 4-OXY-3-METOXYCINNAMIC ACID FROM PROPOLIS

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I. MATEL
CZECHOSLOVAKIA

In order to get a clear biochemical, pharmacological and clinical study of propolis, one has to know exactly its chemical composition. 19 substances with different chemical structures have been identified in propolis so far.

Cinnamic acid and cinnamic alcohol, chrysine (KÜSTENMACHER, JAUBERT) and methylprotocatechnic aldehyde (DIETRICH) are described in propolis. The Soviet researcher POPRAVKO has found isovanillin, acacetyne, kempheryd, rhamnocitryne, quercetyne, pinostrobyne, 5-oxy-7, 4-dimetoxyflavonone, 5,7-dioxy-3,4-dimetoxyflavone, 3,5-dioxy-7,4-dimetoxyflavone and 5-oxy-7,4-dimetoxyflavone.

We isolated and identified caffeic acid and some French researchers, under the co-ordination of V. R. VILLANUEVA, have identified galangyne, chrisyne, testochrisyne, isolphinyne and pinocembryne.

Preliminary experiments and the results of the paper and thin layer chromatography show that propolis also contains a whole series of compounds, still unidentified. We have tried to identify them in our work.

The analysis was performed in the following way: extraction 1000 g propolis were extracted in 2 l anhydrous benzole, without heat over a period of two days. After filtration the benzole solution was removed by distilling it in a vacuum and the deposit was extracted with 1 l anhydrous ethilic alcohol. The extraction took 24 hours without using heat. The reddish-brown solution obtained, was then distilled in order to completely remove the liquid and then extracted in 500 ml cold water, over a period of 3 days. After extraction the deposit was crystallized again in hot water. The crystals separated and dissolved in 10 ml anhydrous alcohol.

The solution obtained was placed on a thin layer of silica gel and was also subjected to chromatographical test using benzole : dioxane : acetic acid system in a ratio of 90 : 5 : 4. By means of UV rays it was established that the chromatogram contained 5 compounds.

The isolated caffeic acid had strips corresponding to a $Rf = 0.24$. In the $Rf = 0.50$ zone, a substance with a blue fluorescence under UV rays was found; this zone was extracted with ethil alcohol vapour extracted and crystallized.

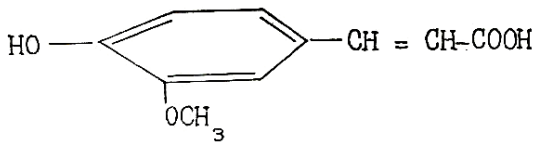
Out of the 15 zones defined in this way, the alcohol extract was obtained by drying. The small crystals resulting after recrystallization in hot water, had a 168°C melting point. Since such a melting point

has not yet been found in propolis, we concentrated on the identification of the substance.

Identification. The alcohol solution of the isolated substance had chemical reactions characteristic for carboxyle and hidroxyle groups with double links and a reaction which demonstrated the presence of the metoxy group.

When examining the alcohol solution under UV rays, we determined a maximum absorption at a wave length of 322 Å.

After studying this substance by means of paper and thin layer chromatography and also through the use of infrared light and the magnetic nuclear spectrum, we established that it was 4-oxy-3 metoxy cinnamic acid which is chemically known as ferrulic acid. It has the following structural formula :



The accuracy of the supposition has been verified by examining the Rf value of the isolated acid and the control acid solution. Both substances had the same Rf values, the same absorption characteristic of the infrared and UV rays.

Pharmacological effect. Ferrulic acid is characterized by an antibacterial effect (gramme-positive and gramme-negative organisms). It contributes to the bactericidal and bacteriostatic effect of propolis.

Besides, it manifests agglutinant effect to a great extent. This feature is made use of in the treatment of difficult wounds, by the help of an ointment prepared from an alcohol propolis solution. Also important is its collagenic effect described in 1938.

Ferrulic acid is to be found usually in the milky juice of the roots of *Ferula foetida,* in the resin of *Opoponax chironim, Catalpa ovata* and in *Ajugaiva.* It also exists in *Equisetum himolo, Dahlia variabilis* and *Berberis amurensis.* Ferrulic acid permeates propolis, especially propolis from the resin of *Pinus laricio* in which it was found in 1876, from *Pinus cembra* and from spruce fir resin.

Bees visit and forage the resin of these plants very often — mainly in autumn. Bees can also synthetize this acid as a by-product from the dissociation of the plant glycosides where ferrulic acid is present in the form of aglucon.

Taking into account the fact that this unsaturated acid was not found in pollen, its presence in propolis is a main reason for attacking KÜSTENMACHER's theory and for supporting the opinion that bees collect propolis from resinous secretions on the buds and bark of different plants.

RESEARCH ON WAXES EXISTING IN PROPOLIS

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Propolis is the glue the bees use for sealing the cracks in the hive and for polishing comb cells. Although it is widely used, its composition has been little investigated. According to previous investigations, propolis was assessed to contain mechanical impurities, resins, and waxes. Two kinds of wax — according to their solubility, are assumed to exist. It was also stated that wax is an indispensable component of propolis, although its proportion depends on the region where bees forage, the method of collection, and on other factors.

Recently, communications have been published on extraction and identification of a number of flavonoids in propolis collected in the European zone of USSR (POPRAVKO) and in France (VILLANUEVA). KELLER and PRUDNICHENKO report in their paper about two kinds of wax extracted from propolis samples from Voronezh region — apart from bees wax — and specify their characteristics.

This paper presents the results of the extraction and determination of a number of physico-chemical characteristics of waxes existing in the propolis collected in Tiumen and Novosibirsk regions and in the Latvian SSR.

Propolis was processed in hot ethyl alcohol in 1 : 10 solution, until the extract was no more coloured. Then, the extract was filtered at high temperature in order to remove the mechanical impurities and the insoluble matter. The insoluble residue was dried until a constant weight was reached. Then the filtrate was cooled down to 18°C, and the No. 1 wax was obtained by straining, which was recrystallized from 96% alcohol until luminescence disappeared in the solutions when analysed by chromatography in ultraviolet light. The mixed extracts were evaporated in vacuum down to their initial volume, and then they were diluted with 70% water. The No. 2 wax was thus obtained, which was recrystallized just as the No. 1 wax.

During the process of recrystallization of wax No. 1 (soluble in hot 96% ethyl alcohol) and wax No. 2 (soluble in hot 70% ethyl alcohol), waxes No. Ia and No. IIa were also obtained, which differ from the previous ones by their specific weight (they conglomerate at the surface), and their solubility in alcohol (wax No. 1 is not soluble in hot 96% ethyl alcohol, and wax No. 2 is not soluble in hot 70% ethyl alcohol).

The final extract was evaporated in vacuum until it dried. As it contains all flavonoids existing in propolis we called it the sum total of flavonoids.

Waxes were saponified with alcoholic solution of alkali by the relevant method (LOKTEV 1970). We determined the content of non-saponifiable substances.

The acid and iodine coefficients were determined by the common methods. The results of our experiment are shown in tables 1 and 2.

For comparison, in table 2 are given data referring to common beeswax from Tiumen region, beeswax which was analysed under similar conditions as the waxes found in propolis.

The investigation of propolis from the three regions of USSR, mentioned above, showed that the product includes 4 kinds of waxes, differing from one another in terms of specific weight and solubility in ethyl alcohol. The total amount of waxes did not exceed 30%. When separating waxes from propolis by sedimentation from alcohol solutions, they also drew in many substances of a flavonoid nature; that is why repeated recrystallization in alcohol was necessary, until no luminescence was recorded in the chromatograms of the extract in ultra-

Table 1

WAXES FOUND IN PROPOLIS FROM VARIOUS ZONES

Propolis sample from	Dry residue %	Total waxes %	Wax No. 1 %	Wax No. 1a %	Wax No. 2 %	Wax No. 2a %	Total flavonoids
Tiumen	11.0	21.0	5.0	12.0	1.0	3.0	65
Novosibirsk	6.0	29.0	4.8	20.0	1.0	2.9	62
Riga	11.5	16.0	2.5	11.5	0.3	1.9	70

Table 2

CHARACTERISTICS OF WAXES FOUND IN PROPOLIS

Kind of wax	Colour	Melting temperature °C	Acid coefficient (mg KOH)	Iodine coefficient	Non-saponifiable substances %
Beeswax (common)	Yellow	64—66	15.0	—	67.0
		TIUMEN			
No. 1	White	62—65	2.4	3.0	15.7
No. 1a	White	60—63	9.3	5.4	93.0
No. 2	White	58—60	3.5	7.5	49.2
No. 2a	Dark Yellow	—	1.4	42.0	53.0
		NOVOSIBIRSK			
No. 1	Yellowish	61	24.0	10.7	54.0 (4)
No. 1a	Sandy	65	6.2	1.0	45.0 (11)
No. 2	Brown	70	28.0	2.6	50.0 (10)
No. 2a	Dark Yellow	69—73	14.4	63.5	46.0 (38)
		RIGA			
No. 1	White	59—60	10.5	1.3	76.0 (28)
No. 1a	White	68—70	11.0	6.0	79.0 (14)
No. 2	Brown	58—59	9.8	3.0	54.5 (38)
No. 2a	Dark Yellow	57—58	11.4	55.0	60.0 (54.7)

*) The quantity of non-saponifiable substances soluble in ether are shown in brackets.

violet light. Most of the recrystallized waxes were white or dark brown; all waxes soluble in 70% ethyl alcohol were dark yellow. The waxes found in propolis are scarcely different from one another in terms of their melting point (50°—70°). However, the melting point of the waxes which are less soluble in alcohol is usually higher.

In all waxes, except wax No. 2, the iodine coefficient is low (1—10), which shows the low contents of non-saturated bonds in hydrocarbons radicals. In all samples of propolis investigated, the wax No. 2 was found to have a relatively high iodine coefficient (42—63), which means that it contains more non-saturated compounds.

The acid coefficient of all waxes found in the propolis samples from Tiumen and from Riga was lower than that in beeswax. The lowest acid coefficient was recorded in the propolis samples from the Tiumen region. The acid coefficient of the waxes found in propolis samples from the same zone was only slightly different.

The waxes extracted from the propolis samples from Novosibirsk have a darker colour and their acid and iodine coefficient is higher.

A relationship was found to exist between the acid coefficient and the content of non-saponifiable substances.

Characteristic of the waxes with high acid coefficient (from Tiumen and Riga) is the higher content of non-saponifiable substances which is likely to be due to a high content of hydrocarbons.

Our paper testifies to the existence of 4 kinds of wax in the propolis collected in 3 regions of USSR.

THE CHARACTERISTICS OF GEORGIAN PROPOLIS

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Research on propolis was directed towards the following objectives:

1. The study of the main palatable and physico-chemical parameters of Georgian propolis;
2. The infra-red spectroscopy of Georgian propolis;
3. Determination of its antimicrobial features;
4. The anaesthetic and immunologic features of this substance.

We obtained the following data:

1. The colour of Georgian propolis is yellowish-green, yellowish-brown, dark brick, dark brown.

The smell of Georgian propolis resembles, in general, propolis from the other areas and regions of U.S.S.R. In some of the samples, a cinnamon or wax smell prevails.

The aromatic samples of propolis came from Armenia, Azerbaidjan and from the mountain regions. Lithuanian propolis has a very strong cinnamon smell. Georgian propolis has a bitter, pricking taste.

The wax content varies between 25—35%. The melting temperature is from 65°C up to 82°C; only three samples had a melting point

over 100°C. The percentage of dissolving in chloroform ranged between 7.64 and 22.44.

Propolis varies in structure and consistency : compact, not compact, hard, and it liquefies at high temperatures.

The oxidation rate of Georgian propolis ranges between 16—21 seconds.

Mechanical impurities are present and range from 18.2% to 33.6% in impurities.

This propolis had a positive reaction as regards the flavonoid compounds. Alcohol propolis solutions, released in combination with ferric chloride, produced a dark green colour with different hues. In a solution of 2% lead acetate, it released a light yellow and orange precipitate.

2. The infra-red spectroscopy of Georgian propolis showed the presence of the following groups of organic radicals :

— double link between carbon atoms, C = C
— the CH group of the furanic nucleus
— the carbonyle group — C = O
— the metoxy group
— the CH_2—O—CH_2 group
— the furanic group
— the ariloxy group, in compounds containing the metoxy group
— the groups — $(CH_3)_2$ C and $(CH_3)_3C$
— the group = C—OH
— cyclic aromatic systems
— aromatic systems
— the δ-lactonic group of butadienoles
— CH_3 and CH_2 group
— CH aromatic group
— OH groups associated with molecular hydrogen
— monooxyflavones which do not contain OH groups in positions C_3 and C_5
— pyrannic rings, with C, Hβ
— anthraquinone with 3 α groups.

Similar groups were also found in propolis other than Georgian. If we had not made the chromatography analysis of propolis samples, we could not have established the specification for the composition of Georgian propolis.

3. When examining the antimicrobial characteristics of propolis, we used cultures of 17 microorganisms and one fungus. We studied the effect of Georgian propolis samples as extracted in vegetal oil and glycerol. We introduced propolis to microbes both in the solid culture media and in a liquid culture medium. We used pure glycerol and pure vegetal oil as controls.

As it can be seen in table 1, in the solid and the liquid medium propolis inhibits the multiplication of the gramme-positive microorganisms — *Staphylococcus albus, Staphylococcus aureus, Streptococcus haemolyticus* etc. in dilution 1 : 50 and 1 : 25. Propolis has also effect

Table 1

Microorganism	Solid culture medium				Liquid culture medium			
	experiment		control		experiment		control	
	propolis in vegetal oil	propolis in glycerol	pure vegetal oil	pure glycerol	propolis in vegetal oil	propolis in glycerol	pure vegetal oil	pure glycerol
B. coli communae	—	—	—	—	—	—	—	—
B. paracoli	—	—	—	—	—	—	—	—
Sh. flexneri	—	—	—	—	—	—	—	—
Sh. stutzeri	—	—	—	—	—	—	—	—
Salmonella typhi	—	—	—	—	—	—	—	—
Salmonella paratyphi B.	—	—	—	—	—	—	—	—
Proteus vulgaris	—	—	—	—	—	—	—	—
Proteus mirabilis	—	—	—	—	—	—	—	—
B. pyocyaneus	—	—	—	—	—	—	—	—
Staphylococcus aureus	1 : 50	1 : 50	—	—	1 : 25	1 : 25	—	—
Staphylococcus albus	1 : 100	1 : 50	—	—	1 : 100	1 : 25	—	—
Streptococcus haemolyticus	1 : 50	1 : 25	—	—	1 : 25	1 : 25	—	—
Streptococcus anhaemolyticus	1 : 50	1 : 25	—	—	1 : 50	1 : 25	—	—
Bac. perfringens	1 : 25	1 : 25	—	—	1 : 25	1 : 25	—	—
Bac. mesentericus	1 : 25	1 : 25	—	—	1 : 25	1 : 25	—	—
Bac. subtilis	1 : 25	1 : 25	—	—	1 : 25	1 : 25	—	—
Candida albicans	1 : 25	1 : 25	—	—	1 : 25	1 : 25	—	—
Sh. sonnei	—	—	—	—	—	—	—	—

against some typical representatives of gramme-negative microorganisms — *Bacterium coli communis, Bacterium paracoli, Salmonella typhi* etc.

In a second series of experiments we verified each sample of propolis (of different origin) on different bacterial strains. We dissolved 2 g of propolis in 10 ml pure alcohol and from this solution I prepared 1 : 10 and 1 : 100 dilutions in saline solution. For the control, I diluted with the same saline solution, 10 and 100 times pure alcohol.

Table 2 refers to samples of Georgian, Lithuanian, Azerbaijan, Armenian and Italian propolis. Out of 16 samples examined, 12 of them were Georgian, the rest were for comparison.

This table shows the number of microbial strains used in the experiments and also the number of strains of a given microorganism destroyed by propolis in 1 : 10 and 1 : 100 dilutions ; we drew the following conclusions :

a) In some cases the propolis solutions hinder the growth of all the strains of a microorganism and in other cases it has a selective effect only on certain strains ;

b) The 1 : 100 solutions are less active than the 1 : 10 ; thus the antimicrobial effect of propolis is in direct relation to its concentration ;

c) Strongly smelling propolis samples have more obvious antimicrobial effect. These samples come from mountain regions (the samples 5, 23, 26, 44).

Number of strains Colour : gramme :		Culture							
		10 +	10 +	10 +	10 —	10 —	10 —	10 —	10 —
Number of sample	*Dilution*	*Staphylococcus albus*	*Staphylococcus aureus*	*Streptococcus haemolyticus*	*Salmonella typhi*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Clostridium perfringens*
(2) Lithuania	10^{-1}	2	—	—	—	—	—	—	—
	10^{-2}	—	—	—	—	—	—	—	—
(3) Georgia	10^{-1}	5	4	6	3	2	1	8	—
	10^{-2}	5	—	2	—	—	—	1	8
(5) Azerbaijan	10^{-1}	10	10	10	5	8	6	8	10
	10^{-2}	10	8	10	5	7	2	6	10
(6) Georgia	10^{-1}	10	10	10	6	9	7	6	3
	10^{-2}	6	9	7	5	6	4	6	1
(14) Georgia	10^{-1}	1	—	—	1	—	—	—	4
	10^{-2}	—	—	—	1	—	—	—	1
(23) Georgia	10^{-1}	10	10	8	—	—	—	—	10
	10^{-2}	10	10	7	—	—	—	—	10
(26) Georgia	10^{-1}	9	7	6	6	10	8	10	10
	10^{-2}	8	3	4	6	9	8	8	10
(27) Georgia	10^{-1}	—	—	—	4	7	7	10	—
	10^{-2}	—	—	—	2	2	6	8	—
(28) Georgia	10^{-1}	10	5	8	—	—	—	—	14
	10^{-2}	6	1	4	—	—	—	—	4
(30) Georgia	10^{-1}	8	10	9	—	—	—	—	11
	10^{-2}	2	10	6	—	—	—	—	8
(31) Georgia	10^{-1}	10	10	10	1	—	—	—	17
	10^{-2}	10	10	10	—	—	—	—	12
(44) Georgia	10^{-1}	10	10	6	—	—	—	—	18
	10^{-2}	10	10	6	—	—	—	—	16
(45) Georgia	10^{-1}	10	10	10	—	—	1	—	4
	10^{-2}	10	10	10	—	—	—	—	—
(52) Georgia	10^{-1}	2	6	8	6	8	14	6	11
	10^{-2}	—	2	4	5	6	10	4	4
(53) Georgia	10^{-1}	1	5	6	4	7	15	5	12
	10^{-2}	—	—	2	1	2	8	2	6
(55) Italy	10^{-1}	4	6	10	—	—	—	—	8
	10^{-2}	1	4	8	—	—	—	—	6

4. I also studied the anaesthetic and immunologic qualities of propolis. I tested the annesthetic ability on the sciatic nerve of a frog. On this occasion I used the Dubois-Raymond apparatus.

We made a 4% dilution (Ringer solution) of 20% propolis alcohol solution. Cotton wool soaked in this solution was applied on the sciatic nerve of the frog for 15 minutes. After that, the numeric calculation of the electric excitation was determined, and also the lower threshold of excitation before and after this treatment. As control, pure alcohol was used, in the same concentration.

In three propolis samples we observed a small decrease in the conductibility of the nervous irritation. It might be thought that propolis has an anaesthetic effect but one has to look for the active principles in propolis ; one should not use the raw propolis but preparations obtained after removing the impurities.

The immunologic characteristics of the samples we worked with were studied by P. SOLOVIOV from the Institute of Sera and Vaccines : he found a slight increase of the immunologic effect on 30 guinea pigs. In this research, they used propolis preparations as adjuvant.

Conclusions

Georgian propolis has well-known palatable, physicochemical and biological characteristics. It has a strong bacteriostatic effect and is also selective on the different strains of a microorganism.

The most active antimicrobial propolis is the strong smelling one, collected in the mountain regions. The anaesthetic and immunologic characteristics of the native propolis are only weakly manifest.

For the use of propolis in greater quantities as bacteriostatic means, we recommend that one takes a mixture of propolis from different zones, because in this case the bacteriostatic activity of propolis on most microorganisms is strongly manifest.

MICROELEMENTS IN APICULTURAL PRODUCTS

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There is a great deal of data in scientific literature concerning the diseases that affect the life of bees, but only few of them show the influence of spur elements from the hive products on human body.

Hive products such as honey, bee venom (apitoxin), royal jelly, propolis, and processed pollen by bees play an essential role in the prophylaxis and treatment of some diseases of man because they contain aminoacids, proteins, balms, carbohydrates, enzymes and what is most important — trace elements.

At present, according to information accumulated by Soviet specialists and others, it has been established that in bee products there are the following trace elements : in honey — aluminium, boron, iron, iodine, potassium, calcium, silicon, lithium, magnesium, manganese, cop-

per, sodium, nickel, tin, osmium, lead, titanium, sulphur, phosphorus, chlorine, chromium, zinc ; in bee venom — iron, iodine, potassium, calcium, magnesium, manganese, copper, sulphur, chlorine, zinc ; in royal jelly — iron, gold, calcium, cobalt, silicon, magnesium, nickel, silver, sulphur, chromium, zinc ; in propolis — aluminium, vanadium, iron, calcium, silicon, manganese, strontium ; in bee bread — barium, vanadium, wolfram, iron, gold, iridium, calcium, cadmium, cobalt, silicon, magnesium, copper, molybdenum, arsenic, tin palladium, platinum, silver, phosphorus, chlorine, chromium, zinc, strontium.

No biological or physiological process takes place in the body of man or animals without the help of spur elements. They are part and parcel of the balance (proteic, fat or glucidic balance) and also participate in the proteic synthesis in the body, in the thermic balance in the haematopoesis, osteogenesis, in multiplication and in immunobiologic reactions.

The human body receives spur elements from food and water. Most of the spur elements contained in hive products were also discovered in the blood and some organs of the human body.

It has been established that there are 24 spur elements in human blood, 22 of them being present in the hive products. If spur elements are insufficient — vanadium, iron, cobalt, copper, manganese, nickel and zinc — the process of haematopoesis is disturbed.

The consumption of these spur elements by means of honey, royal jelly or bee bread, contributes to overcome anaemia.

It is a known fact that spur elements selectively accumulate in different organs of the human body : zinc especially in the sexual glands, hypophysis, and pancreas ; iodine in the thyroid gland ; copper in the liver and in the bone marrow ; cadmium and molybdenum in the kidneys ; nickel in the pancreas ; lithium in the lungs ; strontium in the bones ; manganese and chromium in the hypophysis.

The concentration of spur elements in the blood and tissues is different, its range depending on disease, age and other physiological conditions, even the time of the day and season.

The biological activity of many spur elements is linked with their synergetic action together with enzymes and vitamins. Iron is part of the composition of the breathing enzymes and zinc the composition of the enzymes which make up the glucide and protein balance.

There is a strict dependence between the amount of vitamin B_1 and the manganese content of the body, between the amount of vitamin B_{12} and cobalt. The effect of vitamin B_1 treatment is much stronger if together with food, a sufficient amount of manganese is taken up by the body. During the formation of the bone tissue the presence of cobalt and copper is necessary, the latter being actively linked with the vitamins A, B, C, E and with nicotinic acid.

The physician B. M. HECHT experimented and confirmed that addition of honey, iodine and cobalt intensifies the phagocytosis of white blood corpuscles, increases the resistance of the body to infectious diseases. That is why the regular use of hive products increases the

resistance of the body, not only due to the vitamins contained but also due to the spur elements.

In the case of certain infections, the balance of spur elements is upset in the body tissue. For instance, in the case of endarteritis and in skin affections, a decrease of the copper content in the tissues is recorded. Successful treatment, apart from a complex therapy, depends on the introduction of minor quantities of copper in the organism. It was confirmed clinically and experimentally that minor concentrations of zinc bring about a decrease of colesterole in the blood and the normalization of the balance.

Some liver diseases, hypertonic disease and glaucome cause disturbances to the cobalt balance which is more actively eliminated through the intestine and urinary tracts. By introducing cobalt together with other low blood pressure means (in the case of above mentioned diseases), one finds improvement of the function of the liver and a rapid decrease in arterial pressure.

The above samples demonstrate that spur elements considerably increase the value of hive products and that by being added to the metabolic, fermentative and vitaminic processes of the body, greatly contribute to the efficient treatment of anaemias, prevent the arteriosclerosis, increase immunity to disease and have gerontological characteristics.

III. CHARACTERISTICS OF PROPOLIS

THE ANTIBIOTIC FROM PROPOLIS

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A. Generalities

Propolis is a resin used by the bee to close hive cracks and to ensure its proof against damp and water. The second use is also very important : when the bees kill an enemy in the hive and its corpse is too big to be removed, it is embalmed in propolis which preserves it from decay or subsequent moldiness. The presence of propolis in the wax combs built by the bees must be also noted (it is partly responsible for the colour of the wax).

Propolis could also have, according to some recent works an action over the building of the queen cells *(Vuillaume* 1958). I shall not go into detail concerning the bibliography about propolis, because this is very extended and sometimes it has no scientific basis. I shall only concern with those papers which refer to the antibiotic value, or the medical use of this resin.

It is rather curious that WHITE, who produced a list of the bacteria flora to be found in the comb did not study propolis. In fact, even at that time propolis was considered as a cicatrizing and antiseptic substance, both for animal and plant tissue. Regarding its medical use, CAILLAS (1954) quotes from PARVEL's observations who treated wounds with "propolisin vasogene" during the Boer war, (propolis + vaseline) and found a cicatrizing and regenerating action on the tissues. ROOT quotes from 58 surgical cases in which "propolisin vasogen" was used with excellent results concerning asepsis in wounds. KIVALKINA (1948) and HAMBLETON (1950) revealed the bactericidal properties of propolis. VERGÉ (1951), who quotes from the works of these two authors has also verified the bactericidal power of propolis and finds positive results on *Bacillus subtilis, Bacillus alvei, Bacillus prodigiosus,* and a great sensitiveness of the *white staphylococcus* and the *golden* one as well as of the *pyocyanic bacillus.*

On the other hand, he does not show that propolis had any action on a whole series of *Salmonella*, a series of *Escherichia* and three of *Proteus*.

The most interesting researches on the antibiotics of propolis are those of the two Czechs : FEUEREISL and KRAUS (1958). These authors demonstrated the activity of several extracts from this substance on a number of tuberculosis bacillus strains. They observed that all heated extracts in Soxhlet in solvents as : petroleum ether, ether, benzene, ethanole, water were bacteriostatically inactive. The extract of propolis cold in ethanole is not inhibitory for *tuberculosis bacillus*, but is inhibitory for other strains studied. A cold water extract (a few days of maceration) had a positive action on *Mycobacterium* tbc.

The antibiotic substance can be extracted quicker by the help of chloroform added to the extracting water. Finally, if propolis is subjected to freeze drying, the freeze dried product maintains an antibiotic action. Propolis contains an active antibiotic for *Mycobacterium* tbc. This substance is hydrosoluble and thermolabile ; its efficiency is not modified by lyophilization.

Before passing on to my own researches, I shall quote from the paper of Mayer whose unpublished theses refers to several fractions from propolis, which fractioning was not pushed sufficiently forward so as to permit the chemical determination of the antibiotic.

B. **Private works**

1. *Material and method*

The propolis harvested in the apiaries of the Bures sur Yvette research Station (either in the Paris area or in the south-east) has always given me uniform results. This propolis was treated in two ways : with water and with ethylic alcohol.

Propolis extraction in alcohol at heat is performed as follows : the ground (crushed) propolis wrapped in a cloth is put to boil during an hour into a flask with a cooling system, the extract is then filtered, evaporated in a double-boiler (water bath) and taken again with warm water. The extraction in hot water is obtained in the same way. I put to boil the mixture of water and propolis in withdrawal for one hour, then I filter and concentrate it on a water-bath. In general, for 50 grammes of propolis, it is necessary to use 11 grammes of solvent. I obtained watery extracts which are active to the same extent, as boiling the propolis only 15 minutes in withdrawal.

At the same time in the majority of the trials the alcoholic extracts proved to be a little more active than the watery ones. The extracts taken again in the watery phase are stable for more months if they are kept in a refrigerator, free of light ; being thermostable, they resist sterilization at 120° for half an hour. In watery extracts *Penicillium* does not develop as I found very often in extracts of bees. The extract has a very pleasant flavour. The pH is almost neuter.

2. *Antibacterial activity*

The reaction of the extracts on *Bacillus subtilis* Caron strain is sure and in certain experimetns it goes up to 196 *subtilis* units.

In this case, 10 grammes of propolis correspond to 38 *subtilis* units. These results prove that a propolis extract has a very strong compared to that of the bee antibiotic. Alcohol extracts are, in general, more than reaction extracts obtained in water. I have also tried the antibiotic obtained from propolis on 12 other bacterial strains so as to compare the reaction with that of the bee antibiotic.

The comparison showed us that it is not the same substance. In order to compare the results, let us consider arbitrarily as 10 the reaction of the two substances on *Bacillus subtilis* Caron strain and let us consider the corresponding values of the other bacterial strains :

For the propolis antibiotic,

— Very interesting reaction on *Bacillus subtilis* Caron strain, *Proteus vulgaris* and *Bacillus alvei ;*

— Activity reduced to half or even less on *Salmonella pullorum, Salmonella gallinarum, Dublin* type *Salmonella, Escherichia coli* B, *Bacillus larvae ;*

— Zero reaction on four strains of *Escherichia coli* and *Pseudomonas pyocyanea.*

For the bee antibiotic,

— The reaction is almost always equal or superior to that found for the propolis antibiotic on *Bacillus subtilis ;*

— Bee antibiotic has a reaction related to 10 on *Escherichia, Salmonella* — Dublin type, *Bacillus larvae, Pseudomonas pyocyanea ;*

— Its reaction is twice as much on *Salmonella pullorum* and *Salmonella gallinarum ;*

— Its reaction is four times greater on *Proteus vulgaris* and *Bacillus alvei.*

The comparison of all these results brings me to the conclusion that the bee antibiotic is very different from the antibiotic substances present in propolis. On the other hand, I have shown that bee antibiotic is difficult to extract with hot water ; that of propolis is more easily handled.

It is possible even now to say that the antibiotic extracted from propolis is twice as active on *Bacillus alvei* as on *Bacillus larvae.*

3. *Comparison with other works*

VERGÉ noted a very sharp sensitiveness of pyocyanic bacillus to propolis ; I did not find any bacteriostatic reaction of the propolis extracts on the *Pseudomonas pyocyanea,* but I found, as he found, a positive reaction on *Bacillus subtilis, Bacillus alvei,* but no reaction or a weak one on *Salmonella, Escherichia coli,* (3 strains), *Proteus* X 19. As for other strains, they were not tested in these two cases and so it is not possible to compare results. Vergé found the same antibiotic reactions of propolis extracts as I did, with the exception of their behaviour on *Pseudomonas*

pyocyanea. In my opinion these differences are partially related to the extraction method which was different. The trials were performed with raw and melted propolis, on a watery extract comparable to ours, on extracts in petroleum ether, ether, alcohol and also on an essence obtained by the drawing of the extract into water vapours in petroleum ether.

The trials were performed on agar plates and not by the dilution method as mine. On the other hand, the conditions were not identical with mine. All Vergé's extracts gave positive results (with the exception of the essence drawn into water vapours).

Kivalkina (after Kvitchenko) found that a microbial culture in contact with a plate of propolis is destroyed in a short time, from 10 minutes to 20 hours, depending on the strains studied. In another series of experiments, the raw propolis is added to the culture *environment* in proportions which vary from 1.5% to 10%.

A certain number of bacteria are destroyed, the Sporogenous bacteria requiring the highest level of propolis (10%). The water extract obtained by heating propolis in its own weight of water in a water bath is also active. In this form, pig rinder pest bacillus is destroyed in 30 minutes, but other bacilli resisted for two weeks. From this author, it is shown that propolis has curative bactericidal properties, but they are very variable depending on the bacteria studied.

Kivalkina measured the bactericide and bacteriostatic value of propolis on some strains of bacteria very different from mine so that I cannot compare my results with hers, except for the *pyocyanic bacillus* (unprecised strains). The author found that the bacillus was destroyed after three hours in contact with melted propolis. As for me, I could not show any antibacterial reaction of the water or alcohol extract of propolis on 3 strains of *Pseudomonas pyocyanea* (the strains 3, 4 and 5 from the Pasteur Institute). Vergé confirmed the results of the Russian author.

Feuereisl's works are difficult to compare with Vergé's and with mine ; in fact, these authors worked especially on tuberculosis bacilli ; Mycobacterium (tbc), the $H_{37}Rv$ strain, Ravenel, B.S.G. and M. 57 a. As a common factor, the substance is extractible and soluble in water. Nevertheless I do not agree with the idea of the thermolabile quality of the antibiotic, this being in my opinion thermostable.

Maybe *Vergé* and *Hambleton* are right when they suppose that different kinds of propolis of different origins have not the same chemical composition and the same antibiotic value. With all the regularity of my results, the existence of more antibiotics with different properties cannot be excluded. We shall see further on, that the origin of propolis is a very complex matter.

4. *The origin of propolis and the probable origin of its antibiotic*

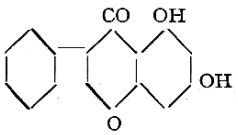
We have seen that the samples of propolis with different origins do not always have a constant antibiotic value. These facts are easily explained by the origin of propolis which should be produced in two ways according to some authors : an external origin — the harvest from the buds and an internal origin — the regurgitation of the resinous substan-

ces coming from pollen. Apart from these two origins, one should remember that propolis is mixed with wax in variable proportions up to 20%, fact which should not be overlooked. In chapter 2 we shall see what the relationship is between these two materials, in relation to their antibacterial reaction.

Bees harvest propolis, or at least a certain resin, from the buds of the trees and they transport this matter in pellets in the baskets on their back legs in the same way as for pollen. This harvesting of propolis was studied by Mayer (1954); it takes place at the warmest period of the day. It has been known from ancient times.

Küstenmacher supported by *Philipp* and *Weck* (quoted by Caillas) admits an internal origin. According to these authors propolis is a residue coming from the first phase of pollen digestion regurgitated by the bee. *Philipp's* works (1923) proved that the drops from the substance taken from the hives would contain exines of little pollen granules and of hairs, which are never to be found in propolis harvested externally. It seems that this propolis would serve for polishing the cells before the egg-laying of the queen. *McGregor* showed (1952) that a bee colony kept in a glasshouse and having pollen was unable to deposit propolis. On the other hand, one holds that propolis comes from poplar buds; but it appears that bees harvest propolis from the buds of many other trees which they find at certain periods of the year. It is a known fact however that the poplar is the most important source of propolis. On the other hand, poplar buds are the only source which produce a residue on extraction with a characteristic propolis smell. *Jaubert* (1927) showed the relationship between propolis and poplar buds. He found a colouring matter, chrysine or 1—3 dyoxyflavone in these two thigs.

Chrysine is also found in wax comb and in honey (Jaubert, 1939). In fact, chrysine is that which gives the yellow colour to wax and honey. This substance is found especially in poplar leaves, in buds, and in the green part of the leaves, 1—3 dyoxyflavone diffuses easily in the fat substances and in the hive it easily passes into wax. This substance represents 0,25% of the weight of the buds, and its extraction is very simple. Chrysine may also be extracted from propolis by the same methods and one obtains pure crystals with the formula:



A proof of the close relationship between propolis and poplar buds was given in the papers of *Vuillaume* (1958). This author showed that propolis extracts and *Populus nigra* buds' extracts, inhibit the construction of queen cells in the bee colony.

To summarise, we may say that many identical substances, smelling, colouring etc., can be found both in propolis and in poplar buds.

C. The presence of antibiotic substances on the plants where the bees harvest propolis

We can compare the antibiotic activity of propolis from the hives with that of substances produced by the trees from which the bees picked up resins. I successively studied *Populus nigra, Abies pectinata, Aesculus hippocastanum, Quercus robur, Pinus sylvestris, Castanea vulgaris.* In addition, I tested different parts of some species of plants : flower buds, leaves, branches, and wood from the log. I knew that the poplar tree contained antibiotics and that bees obtained most propolis from the poplar. Thus we first studied extracts from this tree.

1. Antibiotics in *Populus nigra*

We made extractions either with water boiled in withdrawal for one hour, or with ethylic alcohol boiled in the same way. The liquors obtained are filtered, evaporated and rinsed with water before being added to the culture media, according to our technique based on the dilution method. The water extracts or alcohol ones from buds indicated a similar antibiotic activity and these substances appeared to be very close to the antibiotic obtained from propolis when used on *Bacillus subtilis.* In fact there appears to be 123.9 *subtilis* units for one gramme of dry extract and 10 g of buds contain 55.4 *subtilis* units. The substances extracted from young poplar branches, on the contrary, have less reaction on the same bacillus : I find here three times less *subtilis* units ; an extraction of slivers from the log of a poplar freshly cut does not permit one to obtain an antibiotic substance. The poplar buds extract in water environment looks like of propolis, namely, a related pH, the same smell, and it is preserved for a longer period in a refrigerator. The antibiotic of *Populus nigra* is hydrosoluble, alcohol-soluble and thermostable, the same as the one from propolis. But the resemblance betweeen the two bodies is mainly confined to their antibiotic action on other bacterial strains. One notes a great resemblance in the antibiotic reaction on seven bacteria, in the two extracts (brought arbitrarily to the value U = 10 for *Bacillus subtilis).*

First of all, an equal or superior activity to that obtained on *Bacillus subtilis,* for *Proteus vulgaris* and *Bacillus alvei ;* a weaker action for *Salmonella gallinarum* and *Escherichia coli* B ; a minimal action or even nil for *Escherichia coli* nr. 5512 and *Pseudomonas pyocyanea.* It has to be said that as a whole, the alcohol extract from poplar buds is more active on the different strains studied. The reaction of the poplar buds is very strong against *Bacillus alvei,* pathogen agent of European foul brood. The action of the buds is weak over *E. coli* and *Pseudomonas pyocyanea,* strains on which propolis extract has no effect. I may conclude that the substances extracted from poplar buds are, anyway, present in rough state or slightly modified in propolis harvested by the bees. On the other hand we saw that the other parts of the plants were poorer in antibiotics or they do not even contain any at all. This last

information can be compared with Jaubert's works where he showed that the green poplar organs were richer in chrysine that other parts of the plant.

2. *Antibiotics in some trees of Apis mellifica*

The research of analogous substances on different trees gave me variable results. I never met bodies as active as propolis or poplar buds. The extracts were made in the same way as for poplar organs and in addition by maceration in cold alcohol in certain cases. By heated alcohol extraction one obtains some more active substances than by hot water extraction. But cold alcohol maceration diminishes half of the antibiotic activity of the extracts.

Some of these results are quoted for exemplification in fig. 4 and table 1 ; there emerges some principal data :

Table 1

COMPARISON BETWEEN PROPOLIS EXTRACT ACTIVITY AND BUDS ACTIVITY

Strains	Propolis	*Populus nigra* buds	*Abies* buds	*Aesculus Hyppocastanum* buds	*Quercus robur* buds
***B. subtilis*, Caron strain**	+	− +	+	+	+
***Pseudomonas pyocyanea* 4**	−	+	−	−	+
***Proteus* X 19**	+	− +	−	−	−
Coli bordet	−	−	−	−	−
E. coli 026=B6.Ec. 5434	−	−	−	−	+ −
E. coli					not inves-
055=B5.Ec. 5401	−	−	−	−	tigated
***E. coli* 0111=B4.Ec. 5512**	−	− +	−	−	−
***S.* Dublin type No. 754**	+ −	+	+	+	+
***S. Gallinarum* No. 38**	+ −	+ −	−	+	+
S. pullorum No. 309	+ −	+ −	+	+	+

— All these extracts are less active than the substances present in the *Populus nigra* buds ;

— The *pinus* buds (alcohol extract) are very active on *Bacillus subtilis*, the Caron strain and the antibiotic unit (U = 62,5) is close to that of the resin harvested by *Trigona (Meliponula bocandei)* at Bures-sur-Yvette (U = 73.5).

The oak-tree buds are less active on *Bacillus subtilis* (U = 15.4) than oak slivers freshly cut (U = 41.3). I think that here it is a pheno-

menon owed to the presence of the wood tannin ; the pH of the extract is very acid and it is difficult to harden by cooling the gelosis environment at a strong concentration. In this case it does not seem to be an antibiotic action, but an antiseptic effect due to the tannin.

Wild chestnut buds have a weaker activity than that of the poplar, but this activity is parallel on 10 strains tested.

Fir-tree buds also have a similar activity to that of poplar on 9 out of 10 tested strains, but their extract is not antibiotic on *Salmonella gallinarum* nr. 38.

The extract of oak-tree buds, as opposed to the other plant substances which I tested, is very active on *Pseudomonas pyocyanea 4.*

All these substances of plant origin as well as propolis, are without reaction, or they only have a weak reaction on Enterobacteriaceas of the type Escherichia.

To summarise, there is a safe ratio between the tree buds' resins on the one hand and propolis on the other hand.

At the same time, it clearly appears that poplar buds contain the antibiotic substances most related to those of propolis.

According to my trials, leaf buds, then flower buds, and then little branches, would be the most interesting parts as regards the production of antibiotic substances in these plants, but this is not a general rule. However, how can one explain the fact that propolis always has the same smell as that found only in poplar buds ?

SPUR ELEMENTS OF PROPOLIS AND THEIR BACTERICIDAL VALUE

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Propolis samples collected during 1965—1966 were used for this work. They had been obtained from different regions covering 10 zones of the U.S.S.R. The best methods for obtaining propolis extracts were used. Among the extracts made, the water and alcohol extracts especially are characterized by higher iodine and acid values as compared to these of propolis. There is a connection between the acidity figure and the bactericidal characteristics of propolis and its components on the other.

The correct dose when propolis and its components have bacteriostatic and bactericidal effect was also determined. Propolis as well as the extract in alcohol and ether have an antibactericid effect, especially against grammepositive bacteria. Water propolis extracts have a large spectrum as regards their effect and they also manifest antimicrobial characteristics for grammepositive and gramme-negative bacteria and fungi.

STUDY OF THE ANTIMICROBIAL EFFECT OF PROPOLIS ON THE GASTRO-INTESTINAL MICROFLORA

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The continual use of antibiotics and of sulphonamide preparations often bring about the appearance of resistant forms of pathogenic microorganisms. This urges one to look for new medicines to counteract the side effects of the former.

Research on propolis indicate the efficiency of its use in medicine : dermatology, surgery, otorhinolaryngology, dentistry, and gynaecology. Propolis may also be successfully used in veterinary medicine : for foot and mouth disease, necrobacilosis, ensootic broncho-pneumonia, toxic dyspepsia, staphylococcic mastitis and in rankled wounds.

On the basis of experimental research and of practical use of propolis products, the Institute of Veterinary Medicine at Kazan released "Recommendations as Concerns the Use of Propolis in Veterinary Medicine" (V. P. KIVALKINA, I. F. KAZAKOV, 1962) which stipulate the oral use of propolis as a therapeutic and prophylactic means against gastrointestinal and lung diseases.

In this connection, the study of propolis effect on the microflora at the gastro-intestinal tract is of great practical importance. The complex study of the different characteristics of propolis suggests that it might be used as a raw material in the industrial production of some new natural medicines.

This work has taken into account the study of the influence of propolis in vitro and in vivo on the main representatives of the gastro-intestinal microflora. The bacteriostatic effect of propolis in vitro was tested on cultures of coli bacillus, entrococci, acidolactic bacteria, which are found in the large intestine of animals as well as on staphylococcus cultures (the 209 standard and 39 laboratory strains) and on acidophilous bacteria.

The bacteriostatic effect of propolis that comes from the apiaries of Letonian S.S.R. has not been studied. We studied 9 propolis samples collected from different zones of the Letonian S.S.R. We determined the bactericidal and bacteriostatic effect of the native propolis introduced in the growth media as well as the bactericidal and bacteriostatic effect of alcohol extracted propolis.

I determined the wax content in the raw propolis and of dry matter in the alcohol extract. The results of these researches proved that all propolis has a pronounced antimicrobial effect. The bactericidal effect of the raw propolis, both against staphylococcus and colibacillus, was manifest after 2—4 hours and against enterococcus after 1—3 hours.

The 20% alcohol extracted propolis diluted (1 : 5) in distilled water destroyed the enterococci after 10—15 minutes, staphylococci after 20—30 minutes and colibacillus after 35—40 minutes. In the agar-meat peptone medium the staphylococcus growth ceased after the introduction of

0.05—0.25 ml of 20% alcohol extracted propolis in 100 ml medium; the same, for enterococci — 0.25—0.5, and intestinal bacilli — 3—9 ml. In meat-peptone broth one adds 0.5—1.5 ml, 1.5—2 and 5—10 ml respectively.

The bacteriostatic effect in the agar-meat-peptone was observed in relation with the staphylococcus after inoculation of 0.05—0.025 g propolis in 100 ml medium, with the enterococcus — 0.1—0.5 g and with intestinal bacillus 6—7 g; in the broth of meat-peptone — 0.1—0.25, 0.5 and 7—9 respectively.

The bactericidal effect related to acidolactic bacteria was manifest after the addition of 4—6 g raw propolis in milk, as well as after the addition of 3—6 ml alcohol extract (20% propolis).

The propolis samples contained 1.5—31.1% wax and the alcohol extract — 0.101—0.154 g dry matter for 1 ml. In the alcohol extract prepared from propolis samples with a 1.5% wax content, there was found 0.147 g dry matter in one ml and in the extract obtained from propolis with a 31.1% content — 0.103 g in 1 ml.

The study of the effect of ingested water-alcohol propolis emulsion on the intestinal microflora was performed in 1968—1969 on 39 piglets of the Large white breed, 30 days old and on 20 adult Chinchilla rabbits. They were given 20% propolis alcohol extract orally, for 30 days in the form of 0.5 and 5% water-alcohol emulsion at the level of 4 ml per 1 kg live weight (the 0.5% of the concentration corresponded to the recommendations of the indicated level).

The faeces samples taken from animals every other 10 days, 5 times after giving the preparation, were tested for presence of bacteria — 3 times during the administration of the preparation and twice after it. The number of colibacilli, acidophilous bacteria, enterococci, *Clostridium perfringens* was determined. Apart from the total number of bacteria which were growing in aerobal conditions on agar with meat-peptone, others were determined in tame rabbits.

In the experiments with piglets, 400 faeces samples were tested for bacteria, and 3200 inoculations were performed on rabbits.

In order to see the effect of oral administration of propolis on the gastro-intestinal microflora, a number of coli bacilli and acidophilous bacteria were studied in control and experimental groups, on 30 day and 70 day old piglets; the numbers of bacteria decreased at once. But the number of acidophilous bacteria continued to decrease for the whole period of research. The enterococci and *Clostridium perfringens* contents at different periods of the experiment either grew or diminished. Despite the reduction of the coli bacilli content, these formed the most prevalent group of minor elements until the end of the experiments; the second place was occupied by the acidophilous bacteria and — towards the end of the research — the third place was held by enterococci.

We noticed a wide range of difference in the microorganism content in different individuals within one and the same group growing on the same medium.

The quantitative modifications in the composition of faeces microflora in the experimental piglets and rabbits, revealed by bacteriologic research, did not depend on the effect of the propolis water-alcohol emulsion : they were also found in the control animals. The statistic processing of the data proved that the quantitative modifications in the microorganisms content due to the ingestion of propolis are not significant. This permits us to draw the conclusion that the oral use of the propolis water-alcohol emulsion for a long time does not lead to disbacteriosis, which is of great practical importance.

LITERATURE

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COMPARATIVE STUDY ON THE STAPHYLOCOCCUS SENSITIVITY TO PROPOLIS AND TO ANTIBIOTICS

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Z. PARADOWSKI
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Following recent research conducted in our laboratory we have determined the range of sensitivity of bacteria to an alcohol propolis solution, which proves the efficiency of this solution in the treatment of purulent dermatitis and gynaecological affections.

The antibacterial effect of propolis samples from various sources differ. The method with discs we used for determining the effect of propolis is based on inhibiting the development of a standard strain (*Staphylococcus pyogenes* Oxford 209 P) by means of a certain concentration of propolis. The results of these experiments and the detailed clinical method used have already been published.

During our tests we compared the sensitiveness of staphylococci isolated from pathogen sources with that of the strains in our collection. Our second goal was to prove the possible correlation between the sensitiveness to propolis and antibiotics of the staphylococci isolated from pathogen sources.

Material

We tested 56 staphylococcus strains isolated from pathogen sources. The antibacterial effect of propolis — by inhibition of the development of standard strains, was determined for 3 mg/ml. The sensitivity of staphylococci to antibiotics was determined by the common method of discs. The antibiotics used were penicillin, ampicillin, meticillin, streptomycin, cloramphenicol, terramycin, erythrocyn, myacyn and sulphathiazole.

Results

For a few staphylococci isolated from pathogen material, a sensitivity to propolis was recorded, comparable to that of the standard strains. Of the 56 tested species, only 5 (approximately 9%) were inhibited by propolis in concentration of 3—9 mg/ml, 23 strains (41%) — by a 12—15 mg/ml concentration — defined by us as low sensitivity to propolis, and 28 strains (5%) were inhibited by 18—21 mg/ml concentration — which we defined as great resistance to propolis. We could not state precisely which was the correlation between the sensitivity of the tested staphylococci to propolis and to antibiotics.

All strains whose sensitivity to propolis was certain were highly resistant to the tested antibiotics. The strains with low sensitivity to propolis and various degrees of sensitivity to antibiotics, just as the resistant strains. For the last two groups, a general diminution of sensitivity to antibiotics was recorded — considering the average zone of inhibition.

The results are given in tables 1, 2 and 3.

Table 1

SENSITIVENESS TO ANTIBIOTICS OF PROPOLIS SENSITIVE STAPHYLOCOCCI

Sensitiveness to propolis	Sensitiveness to antibiotics: average inhibition zone of the staphylococci culture, in mm	
3—9 mg/ml (5 strains)	penicillin	13.4
	ampicillin	15.0
	meticillin	15.0
	streptomycin	16.4
	chloramphenicol	15.6
	terramycin	13.0
	erythrocyn	19.0
	myacyn	20.0
	sulphathiazole	13.0

Table 2

SENSITIVENESS TO ANTIBIOTICS OF LOW PROPOLIS SENSITIVENESS STAPHYLOCOCCI

Sensitiveness to propolis	Sensitiveness to antibiotics: average inhibition zone of the staphylococci culture, in mm	
12—15 mg/ml (23 strains)	penicillin	23.9
	ampicillin	23.2
	meticillin	20.1
	streptomycin	24.8
	chloramphenicol	20.3
	terramycin	21.4
	erythrocyn	23.4
	myacyn	27.2
	sulphathiazole	28.6

Table 3

SENSITIVENESS TO ANTIBIOTICS OF PROPOLIS RESISTANT STAPHYLOCOCCI

Sensitiveness to propolis	Sensitiveness to antibiotics: average inhibition zone of the staphylococci culture, in mm	
18—21 mg/ml (28 strains)	penicillin	25.2
	ampicillin	25.2
	meticillin	23.2
	streptomycin	26.7
	chloramphenicol	23.9
	terramycin	21.8
	erythrocyn	24.9
	myacyn	27.4
	sulphathiazole	32.2

Conclusions

1. In most cases, the staphylococcus strains isolated from pathogen materials had a low sensitiveness to propolis; only 10% of the tested strains had a sensitiveness comparable to that of the strains collection. The diminution of their sensitiveness is likely to be caused by the metabolic changes occurring in them, changes which — when the strains are separated from the body — can be proved as having effect of an enzyme. We will make further thorough research in this respect. It is however likely that the *in vitro* sensitiveness of staphylococci to propolis is not exactly the same as their *in vivo* sensitiveness because the tests made in clinics produced better results when propolis was administered in purulent infections.

A similar case is the sensitiveness of bacteria to antibiotics — the results of the *in vitro* tests are not the same as the results of clinical tests.

2. No correlation was found between the sensitiveness of the tested staphylococci to propolis and to the common antibiotics. Noteworthy is the fact that the propolis highly sensitive strains were resistant to antibiotics. Further thorough research in this respect is expected to be taken in the future.

EFFECT OF PROPOLIS ON SOME SPECIES OF MICROORGANISMS AND MOULDS

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A great attention is given at present to the study of propolis not only on the domain of apiculture but also in chemistry and medicine. I was interested in propolis not only as a beekeeper but also as a chemist since I also work in the industry of antibiotics.

Propolis is a natural hive product which — quotation from *Der Imkerfreund* — contains about 55% resinous substances and glues, 10% essential oils, 30% wax and 5% pollen. At cool temperature, propolis is a solid and friable substance and at heat it becomes soft, pliable and adherent. It is also BRENNER's opinion that the colour of propolis is chestnut, reddish or greenish.

The antimicrobial effect of propolis is — according to SMID — strongly influenced by the source it comes from. VILLANUEVA et al. show that the most active part of propolis is represented by galangyne, 3,5,7-trihidroxyflavone respectively. Besides, even since 1927, chrysine and tectochrysine flavones have been known. These flavones are — according to LAVIE — always included in the glues of poplar buds. Thus, the supposition that propolis has its origin in tree buds might be correct.

Description of the experiment

The purpose of the experiment was to draw some conclusions as regards the antimicrobial effect of propolis on some microbs, moulds and yeasts.

According to BERGEY's bacteria classification system the following species were determined : from the *Eubacteriales* order : *Escherichia coli* 9637/37, *Micrococcus flavus* ATCC 10240, *Micrococcus lysodeicticus* ATCC 4698, *Staphylococcus aureus* P — 1485, *Sarcina lutea* ATCC S 341, *Streptococcus cremoris* NIRD 185, *Corynebacterium equi* BU CSAV 184, *Bacillus licheniformis, Bacillus subtilis* BL 750, 2g, *Bacillus antracoides ;* from the *Eumycophyta* subphylum : *Fusarium solani* 257 ; *Aspergillus ochraceus* 511 ; from the yeasts of *Endomycetaceae (Saccharomycetaceae)* family : *Saccharomyces cerevisiae* ATCC 2611 ; from the *Cryptococcaceae* family : *Kloeckera apiculata* BU CSAV and *Candida albicans.*

This work is divided into three parts :

The first part examines the general effects of propolis on the species of bacteria, moulds and yeasts tested. In the second part of the experiment we tried to establish the antimicrobial effect of propolis. In the third part, propolis efficiency was determined as compared to the antibiotic effect of penicillin and fungicidyn.

The propolis used during the experiment came both from the northern zone of Prague and from the Jizerské mountains. As solvent, 96% ethyl alcohol was used. The propolis ethyl alcohol ratio was always 1 : 3 because this proportion ensures an easier dissolving and avoids the saturation of the solution. Propolis with ethyl alcohol was poured afterwards into an Erlenmayer flask with a bulk of 300 ml which had a

plastic cork; the flask was placed in the mixer and it was stirred at the temperature of 31°C for 36 hours, in the dark. The content of the flask was then filtered through a paper filter in order to remove the inert material and the undissolved propolis. The propolis dissolved in ethyl alcohol was preserved in the dark at 12°C. In order to secure the homogeneity of the samples, in each research stage, one single level of propolis dissolved in ethyl alcohol was always prepared. As initial microbial material, the microbes preserved on horse serum, freeze dried were used.

As initial culture liquid medium they used glucose broth. The bacteria were grown for 24 hours at 37°C — the moulds for 10 days at 31°C — the yeasts for 48 up to 72 hours at 25°C. The culture in broth was preserved at cold being always used only for a few days. In order to further preserve it, the culture was inoculated on sloped agar, was grown for a certain period and then kept at —5°C. From the culture thus preserved, the microorganisms may be again inseminated on glucose brothü For the proper experiments we used solid media, agar-blood in most of the cases. Agar was used for the testing of relative efficiency in comparison to the antibiotics. For moulds, we used Sabouraud media.

Instead of the common petri dishes we used plastic ones. The upper half is proped up (in the case of Heatly method) on steel rolls which considerably reduce ethyl alcohol evaporation. The paper filter and the steel rolls were sterilized in a hot air sterilizer at 160°C, for 3 hours.

Results of the Experiment

Part I.

In order to determine a sensitiveness of a microorganism to propolis we used strips of filter paper with the size of 205 mm and also washers with the diameter of 10 mm. The sensitiveness was experimented for all the 15 species of minor elements subjected to the test. The filter papers and the washers have been wetted with the propolis solution in ethyl alcohol so that no solution should drip on the solid medium. Alongside with the sensitiveness to propolis there was also determined the sensitiveness of the different cathegories of minor elements to the ethylic alcohol. The result was negative.

Part II.

The majority of the microorganisms in the first part of the experiment were also used in part II, when they established once more the

Table 1

RESULTS OF THE SENSITIVENESS TEST

Escherichia coli	+
Micrococcus flavus	++
Micrococcus lysodeicticus	+++
Staphylococcus aureus	+++
Sarcina lutea	+
Streptococcus cremoris	+++
Corynebacterium equi	++
Bacillus licheniformis	+++
Bacillus antracoides	+
Bacillus subtilis	+
Fusarium solani	+
Aspergillus ochraceus	+
Saccharomyces cerevisiae	++
Kloeckera apiculata	+++
Candida albicans	+++

+++ — maximum sensitiveness
++ — medium sensitiveness
+ — minimum sensitiveness

antimicrobial effect of propolis. The efficiency of propolis was found by help of the Heatly method, that is, with steel rolls having the diameter of 7.5 mm and by inducing inhibition zones.

The results are included in table 2.

Table 2

Species	Inhibition zone, in mm				
Micrococcus lysodeicticus	12.0	10.0	10.0	9.2	9.0
Staphylococcus aureus	12.4	9.5	10.0	10.2	8.8
Sarcina lutea	10.0	12.0	9.2	10.5	11.0
Streptococcus cremoris	9.0	9.2	0	9.5	8.8
Corynebacterium equi	9.0	13.0	10.3	9.5	11.1
Bacillus licheniformis	10.0	9.0	9.5	8.0	0
Bacillus subtilis	10.8	12.2	11.8	13.5	11.5
Candida albicans	9.0	8.0	9.0	0	8.5
Fusarium solani	8.8	13.0	14.0	10.2	11.0
Aspergillus ochraceus	10.0	12.0	12.5	12.5	12.5
Saccharomyces cerevisiae	11.0	10.0	9.8	9.5	0
Kloeckera apiculata	10.0	12.0	9.5	9.2	0

The propolis concentrations in ethyl alcohol were tested on *Bacillus subtilis, Sarcina lutea* and *Staphylococcus aureus.* The results are given in table 3.

Tabel 3

Species	Propolis concentration in ethyl alcohol				
	1 : 1	1 : 3	1 : 4	1 : 6	1 : 10
Bacillus subtilis	10.0	10.0	11.0	10.0	9.5
Sarcina lutea	10.0	11.0	10.0	10.0	8.5
Staphylococcus aureus	10.0	10.0	10.0	9.8	9.8

In order to induce a better diffusion of the propolis dissolved in ethyl alcohol after the addition of water, experiments were performed on *Saccharomyces cerevisiae, Staphylococcus aureus, Corynebacterium equi* and *Sarcina lutea.*

For 9 ml of propolis solution in ethylic alcohol (1 : 3 ratio) 1 ml of distilled water was added. The results are rendered in table 4.

Table 4

Species	Inhibition zone, in mm			
Saccharomyces cerevisiae	10.8	10.8	11.0	11.5
Staphylococcus aureus	10.0	10.0	10.2	9.5
Corynebacterium equi	10.5	10.0	10.8	10.5
Sarcina lutea	10.5	9.8	10.0	10.8

The thermostable characteristic of propolis was experimented by heating the propolis dissolved in ethyl alcohol (1 : 3), on a water bath, in four ampoules heated at the temperature of 40°C, 60°C, 80°C and 100°C respectively. The heating lasted three minutes. The results may be examined in table 5.

Table 5

Species	Temperature, °C			
	40	60	80	100
Staphylococcus aureus	10.00	10.00	9.0	9.5
Sarcina lutea	11.00	12.00	11.2	11.0

Part III.

In this section of the paper one establishes the relative effect of propolis as compared to some antibiotics. For penicillin and propolis, *Staphylococcus aureus* was used as microorganism for testing. The result was negative, because a resistant strain might have been tested.

Propolis created inhibition zones but not penicillin. This is why *Bacillus subtilis* was used. The experiment was performed on a wider dish, with endoagar, in which the culture of microorganisms grown in broth was again inseminated.

7 rolls with propolis dissolved in ethyl alcohol were placed on endoagar (1 : 3) and penicillin 16, 8, 4 u.

The culture was kept in thermostat at 37°C. The results are presented in table 6.

Table 6

Substance used	Inhibition zone, in mm						
Propolis in ethyl alcohol	12.2	14.0	13.5	14.0	12.5	12.5	12.8
Penicillin, 16 u.	12.2	12.4	12.3	12.4	12.6	12.5	12.4

For determining the propolis effect as compared to the effect of fungicidine, the yeast *Saccharomyces cerevisiae* was used as microorganism for testing.

The concentration of propolis in ethyl alcohol was 1 : 3 ; fungicidine — 100 u, 50 u and 25 u. The results are shown in table 7.

Table 7

Substance used	Inhibition zone, in mm			
Propolis in ethyl alcohol	12.2	12.6	12.5	12.4
Fungicidine 100 u.	20.0	20.0		
„ 50 u.	17.3	17.0		
„ 25 u.	12.5	12.5		

Results and discussions

In this work we wanted to stress the antimicrobial effects of propolis on some species of microorganisms.

Its antimicrobial effects were demonstrated by a great number of authors.

LINDENFELSER considers that the general bacterial and fungic effects of propolis were revealed especially in the grammepositive bacteria. The propolis did not influence any of the two yeast cultures tested.

From the experiments there results that propolis has an antibiotic effect on the majority of the minor elements tested, mainly on the grammepositive cocci — *Micrococcus lysodeicticus, Sarcina lutea, Staphylococcus aureus,* also on the grammenegative bacilli — *Bacillus subtilis,* on grammepositive bacilli — *Corynebacterium equi* as well as on some mould species — *Aspergillus ochraceus* and on some yeasts — *Saccharomyces cerevisiae.*

The various propolis concentrations in ethyl alcohol did not present essential differences — up to the 1 : 10 concentration which had the lowest efficiency.

There was no difference between the effect of propolis dissolved in ethyl alcohol and that of the propolis in ethyl alcohol solution with addition of water.

From the experiments performed with the effect of testing the thermostable characteristic of propolis, we did not notice significant differences. We may suppose that propolis is a thermostable substance. This feature was also revealed by KIVALKINA.

The relative antibiotical effect of propolis corresponds to the value of 16 u. penicillin and 25 u. fungicidin.

It is worth mentioning that the results of the tests could be influenced negatively by one of the features of propolis, that is, its characteristic of being more soluble in alcohol than in water. On the other hand, although the evaporation of the ethyl alcohol from the rolls was avoided (since the upper half of the Petri plates was supported directly by the rolls), this process could take place due to the diffusion of the solution in solid medium.

This is why we can explain the negative effect recorded when testing the ethyl alcohol on the species of microorganisms on which we made experiments. The titration methods could not be exploited very precisely, because even a minimum concentration of ethyl alcohol in liquid culture media has antimicrobial effect.

On the occasion of these experiments, many questions whose answer would be given by subsequent experiments, were formulated. In the future we intend to test the effect of propolis on a larger sphere of microbial spectrum in order to investigate this substance pharmacologically.

THE INHIBITORY EFFECTS OF PROPOLIS ON SOME PLANT VIRUSES

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V. KOSLJAROVÁ
CZECHOSLOVAKIA

Propolis is used by bees for tightening the hives, levelling and polishing of the comb cell walls, propolization of the animals or of dead insects on the bottom or on the walls of the hive. In its production different glues resins and colouring substances also contribute. They are collected by bees from nature and carried to the hive as well as undigested particles of pollen grains, of wax and of mineral substances.

Propolis has different colours : green, chestnut, brown, yellow-brown, dark brown up to black. Propolis is more compact than bee wax and, placed in water, it deeps into it. It is insoluble in water and only partially soluble in alcohol ; it dissolves easily in ether and chloroform. At 15°C it is hard and pliable. At higher temperatures it melts and it becomes sticky. The melting point is at 60—69°C. Its chemical composition is not yet searched enough. In general one may assert that propolis contains glues, resins, and other substances.

The propolis has strong bacterial and bacteriostatic effects. Tiny corpses from the hive wrapped in propolis do not decay. The effect of propolis is due to a great extent to its chemical composition. Its anaesthetic effects are exceptional : 3.5 times greater than those of cocaine and 5.2 greater than those of novocaine. For these qualities, propolis is largely used in veterinary and human medicine. (CURYLO, 1970).

In vegetal virology where there comes the problem of pathogenic agents, there is no information referring to experiments with propolis. Usually they would use inhibitors — substances which hinder infection or reduce the multiplication of the virus in the cells of the affected plant. They are both organic and inorganic substances, chemically pure as well as complex substances, undefined from the chemical point of view.

Among the latter there are especially different plant extracts, the treatment with curd milk etc. We could classify propolis among the inhibitory substances.

Method and results

For our experimental purposes, we obtained propolis from a beekeeper from Bratislava. We prepared a 10% solution, stirring a flask with 25% ethanole and propolis, for 30 minutes. The solution was left to rest for 24 hours, then it was stirred again and filtered through gauze, in order to remove the rough impurities. After settling, a dark chestnut colour was found. The propolis solution thus obtained was preserved at dark, at a temperature of — 3 to — 9°C.

For the experiments with propolis we used three species of virus : the cucumber mosaic virus, isolated from *Phytolacca americana* (KOSLJAROVÁ, BOJŇANSKY, 1972), the tobacco spotting virus *(Nicotiana virus 12* [Fromme] Smith) and the tobacco necrosis virus, isolated from *Evonymus europaea* (BOJŇANSKY and KOSLJAROVÁ, 1968). For the first works we used a 10% propolis solution in 25% ethanole.

In the first experiments we used different ways of application as follows :

a) the inoculation of white beans leaves *(Phaseolus vulgaris)* with the cucumber mosaic virus or with the tobacco spotting virus and even the inoculation of cucumber leaves (*Cucumis sativus* cv. delicates) with the tobacco necrosis virus. After 5 minutes — the applying of a 10% propolis solution on the inoculated leaves ;

b) the application of a 10% propolis solution on the beans leaves or on the germinative leaflets of the cucumber, and after 5 minutes — inoculation of the corresponding virus ;

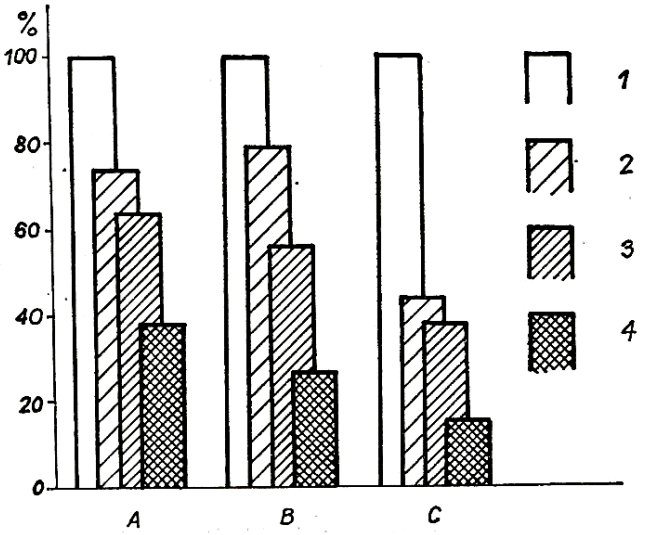
c) the mixture of the virus inoculum with a propolis solution of 10% in the ratio of 1 : 1 ;

d) the control virus inoculum, without the use of propolis.

The results obtained from these experiments appear in graph 1.

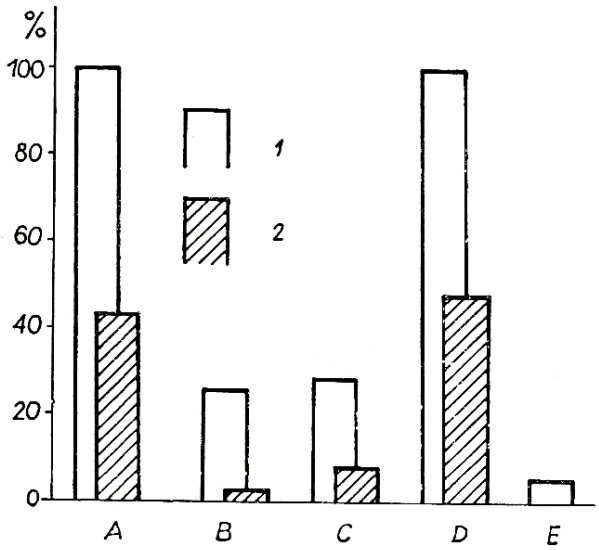
The most reduced sensitiveness was revealed by the cucumber mosaic virus and the highest for the tobacco necrosis virus. The procedure of virus inoculation + subsequent application of propolis have shown minimum efficiency. In the case of this procedure the number of lesions on the leaves was reduced by 20—55% as compared to the control. More efficient proved to be the applying of the virus mixture + propolis solution on the bean leaves and on the germinative leaves of the cucumber, by the same operation, immediatly after the mixture of the two components.

By this procedure, the number of lesions was reduced with 36—62%. The maximum effect was ensured by the application of propolis solution on the leaves of the plants tested followed at an interval of 5 minutes by inoculation with the corresponding virus. In this case, the number of lesions decreased with 62—85%.



Graph 1 — The impact of the different ways of propolis application on some viruses : A — the cucumber mosaic virus; B — the tobacco spotting virus; C — the tobacco necrosis virus. The 4 columns from right to left: control; inoculation of virus and application of propolis after 5 minutes; mixture of virus and propolis; application of propolis and inoculation of the virus after 5 minutes.

In the case of the second experiment I used the virus with the greatest sensitiveness, that is the tobacco necrosis virus as well as the most becoming procedure of application of a 10% propolis solution on the germinative leaflets of the cucumber and then the inoculation with virus. In comparison to the control I obtained a reduction of about 57% (the 2 A graph). The tobacco necrosis virus has provoked necrotic lesions on the germinative leaflets of the cucumber with a pronounced local character and easy to be counted, but the virus penetrated the whole plant. By this, we intended to verify how quickly the virus reaches and is multiplicated in root vines, leaflets and cucumber leaves. To this purpose we made a new inoculation (after 17 days) by using the juice extracted from different parts of the plantlet both from the control material and from that to which the propolis was administered. The results of the test are illustrated in graph 2.



Graph 2 — The influence of propolis on the reproduction of the tobacco necrosis virus in different parts of the plantlets (cucumber)

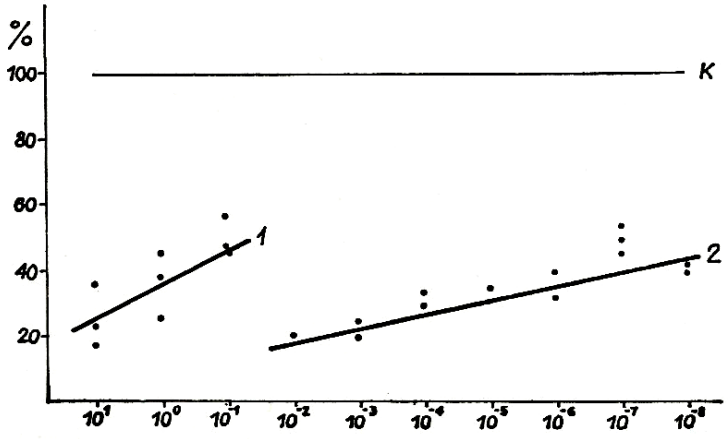
A — the number of lesions on the cucumber ovary
B — the concentration of the virus in the plant
C — the concentration of virus in the plant vines
D — the concentration of virus in the germination leaflets
E — the concentration of virus in leaves
The stripped columns : material treated with propolis ; the white columns : control material.

In the roots of the material treated with propolis there was registered a quantity of virus of about 12 times less than in the germinative leaflets of the control material. In the true leaves of cucumber, the presence of the virus was registered no more — in the case of the materials treated with propolis, while in the control material it appeared even only in short quantity, that is about 5% as compared to the germinative leaflets of the control material.

The third experiment was also performed with the virus of tabacco necrosis on the germinative cucumber leaflets. The purpose of the experiments was to determine the dilution possibilities of propolis and maybe the decrease of the propolis effect depending on the increase of the dilution.

The first part of the experiment was performed with bigger concentrations, that is 10%, 1% and 0,1% in three successive series. The second part of the test was performed with inferiour concentrations, that is 10^{-2} up to 10^{-8} in two successive series. The results appear in graph 3. In comparison to the control material we obtained in almost all cases a diminish under 50%.

In the fourth experiment we used the classical virus of tabacco mosaic which presents viral particles under the form of a stick. Two

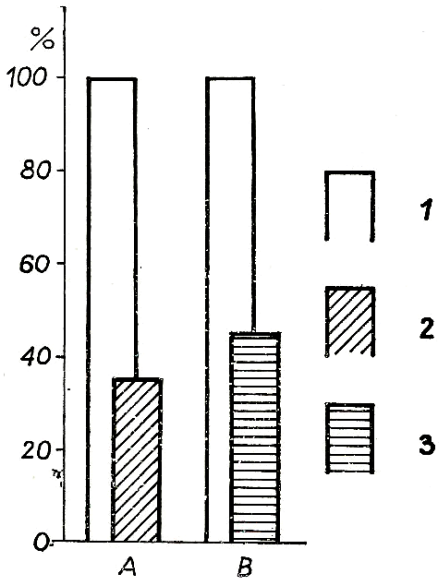


Graph 3 — The inhibitory effect of propolis on the constitution of lesions after the inoculation of the tobacco necrosis virus on the germinative leaflets of the cucumber
On the ordinate, the percentage of lesions in comparison to the control material; on the abscissa, the percentage of propolis concentration. K — control material ; 1—2 — material treated with propolis

species of tabacco were used, that is *Nicotiana rustica* and *Nicotiana glutinosa* on whose leaves, the inoculated virus forms necrotic lesions easy to be seen. In *N. rustica* the leaves were treated with a propolis solution in the concentration of 10^{-5} and after an interval of 24 hours tobacco mosaic virus was inoculated. In the case of *N. glutinosa*, the virus was inoculated only after the passage of 48 hours. The results appear in graph 4.

In the first case of this series of experiments, the number of lesions decreased by 65.5%, in the second case — by 56.3 which corresponds (taking into account the late inoculation of the virus after propolis administration) to the inhibitory effect of propolis on the spheric virus of the tobacco necrosis.

In the fifth experiment I verified the sensitiveness of propolis, the maintenance of its inhibitory effect on the plant virus, after different duration and intensity heatings.



Graph 4 — The inhibitory effect of propolis (in the concentration of 10^{-5}) on the tobacco mosaic virus. On the ordinate, the percentage in lesions, in comparison to the control material

A — *N. rustica:* the stripped column — material treated + after 24 hours VMT ; the white column — control

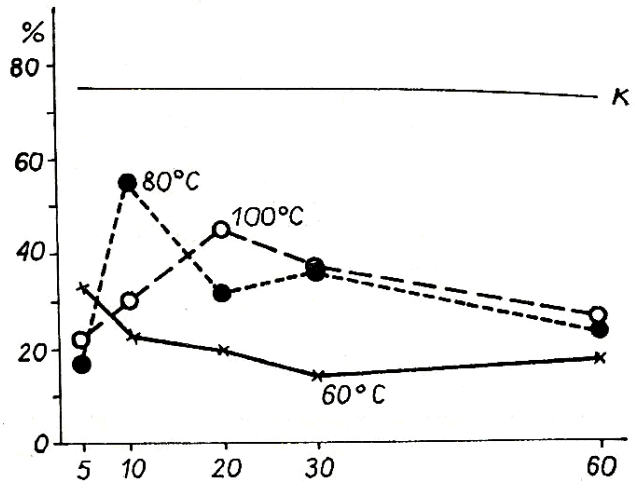
B — *N. glutinosa:* the stripped column — material treated with propolis + after 48 hours VMT ; the white column — control

During the experiment they worked with two virus, the tobacco necrosis virus and that of spotting tobacco, by using 60°C, 80°C and 100°C temperatures, for 5, 10, 20, 30 and 60 minutes.

The propolis in the concentration of 10^{-5} was heated for a certain period of time at the proper temperature in ampoules with thin glass on a water bath and before administration it was cooled under a jet of water. As control untreated plants with propolis were used. The results of the experiments are illustrated in graph 5.

The efficiency of propolis was affected to a very small extent at the temperature of 60°C and it was maximum affected at the temperature of 100°C. In the virus of the spotting tobacco, the short term heating (20 minutes) at the temperature of 80 and 100°C has reduced the efficiency of propolis more than the heating of it for 60 minutes.

In the case of the tobacco necrosis virus a similar effect was observed at the heating (at 80°C) for 10 minutes, maybe for 20 minutes (at 100°C). One can assert finally that propolis preserves to a great extent the inhibitory effect on virus, even after having been subjected for a long period to high temperatures, which means that its inhibitory effect has a great stability.



Graph 5 — Influence of heating on the propolis inhibitory effect. K — control
On the abscissa — time of propolis heating in minutes, on the ordinate — percentage of lesions on a leaf as compared to control

Discussions

Up to the present time there were no informations about the use and experimentation of the propolis effect on the plant virus.

We suppose that our results are the first information in this respect.

The propolis used has demonstrated its inhibitory effects on the all four phyto-virus experimented and this is in a great proportion. Three virus species have spheric particles but, despite of this resemblance, their sensitivity is different.

The greatest sensitiveness was demonstrated by the tobacco necrosis virus and the most reduced — by the cucumber mosaic virus, that is, by its strain isolated from *Phytolacca;* the fourth virus has particles under the form of sticks and it seems that its sensitiveness to propolis is almost similar to that of the tobacco necrosis spheric virus.

The results included in graph 2 clearly demonstrate not only that propolis reduces the number of lesions on the virus inoculated leaves, but also it obviously inhibits the replication of the virus in the whole plant.

For the time being it is not possible for us to explain the greatest effect of the reduced concentrations (optimum results were obtained with the concentrations of 10^{-3} — 10^{-7}). These cases were the effect either of physiological status of the plants (the experiments being performed successively not simultaneously), of the medium, or of another uncontrollable factor. According to the last experiments we can draw the conclusion that the propolis maintains its efficiency even in very reduced concentrations, fact which could present a special practical importance in the case of its use when having in view to protect the most sensitive cultures especially the glass house cultures young siblings and plant beds. The inhibitory effect of propolis on viruses has a remarkable stability which is also maintained to a great extent in the case of heating to high temperatures.

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STUDY OF THE LOCAL ANAESTHETIC CHARACTERISTICS OF PROPOLIS AND THEIR EFFECT IN OPERATIONS ON SHEEP AND DOGS

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Up to the present time the origin of propolis has not yet been precisely established (8). It is a resinous substance, of a dark-green colour and it contains resins, balsams, wax, etheric oils and pollen. The propolis has the vitamins A, B, E and P.P. (2, 3).

It easily dissolves in ether, ammonia and alcohol (7). Due to its antibacterial characteristics and to the acceleration in creating a new combination, the propolis finds a widespread application in medicine (1).

In our researches (9) the propolis was used as a means of anaesthesising the pains in the operations on the abdomen in domestic animals.

In this research we proposed the study of its anaesthetic and general action in experimental operations on abdomen in sheep and dogs.

Research Material and Methods

The propolis we have used was collected in 1970 from a village and is good to our needs, according to KIVALKINA (6). I used alcohol and hydroalcoholic extracts prepared on the spot.

I obtained a propolis watery extract and distilled water in the ratio of 1 : 1 by heating it in a water bath for one hour.

I similarly prepared the 30% hydroalcoholic extract out of 26 ml alcohol (95°), 84 ml water and 33 g ground propolis. After filtration and sterilization the propolis was administered intramuscularily and under the skin as well as for the superficial anaesthesia of the abdominal organs.

The alcoholic extract is prepared as follows : some ground propolis in 1 : 4 ratio in 95° alcohol stays thus for one hour. 5 ml of the extract thus prepared and filtered correspond to 0.150 g dry matter.

By the researches performed we established (9) that the level of efficient dry matter is 0.012 g/kilobody given orally.

We made research on 12 adult sheep of local breed. Merinous for meat having a weight of 35.40 kg and on three male dogs of impure breed with the weight of 8—10 kg.

All animals were clinically healthy. I controlled their healthy before and after the operation three times a day. The blood examination, in order to find the surgical modifications and those due to the anaesthesy, was performed once before and several times after the operation.

I observed modifications in the number of erythrocites and white cells in their aspect in the haemoglobin quantity (according to Sahli)

in the VSH and in the proteic fraction of the serum (by microelectrophoresis on agar gel).

We performed the operations with the respective anaesthesy: a) in 4 sheep we did the subcutaneous punction. Two of them were given 25 ml alcoholic extract orally with tranquillising effect and two of them were control; b) other two sheep were given 30 ml watery extract intraperitoneally.

They were also made subcutaneous punctions; c) in 6 sheep the laparotomy was made on the left side (fossa paralumbalis).

Three of them were injected in the incision area for local anaesthesia, with 15 ml of propolis hydroalcoholic extract and the others with 15 ml novocaine solution (5%); d) in the dogs immobilized, a part of the intestine was eradicated.

I injected 20 ml watery extract in the anaesthesy for laparotomy. I used this extract also for the superficial anaesthesia of the operated organs and of the peritoneum. I did a similar operation, only in the control animals (on dogs) when a mixture of 1 : 1 alcoholic extract combined with novocaine was used (0.25%).

Results and Opinions

In the oral administration of the propolis alcoholic extract I observed that after 15—20 minutes, an efficient anaesthesia begins. In the case of punction all sheep manifested pain and a refraction while when exciting the mucous membrane of the skin or of the peritoneum when I introduced in the trocar canula a hard tube, the anaesthetised sheep (in contradistinction to the control ones) had no reaction.

The sheep treated with the introduction of a propolis watery solution in the abdominal cavity and excited by the above mentioned procedure, reacted in one hour much the same way as it given the solution orally.

In the sheep in which we did the abdominal incision after the local infiltration the effect of the anaesthesia was manifest after 2—5 minutes from the application and it lasted 45 minutes.

I saw no difference between the anaesthetic effect and that of novocaine.

In dogs, the anaesthesia began at 2—5 minutes since the application and it lasted approximately 45 minutes.

At the end of the operation we irrigated supplementarily the stomach and intestines with propolis watery extract in order to reduce the pressure, to ease the position of the intestines and the cicatrization of the abdominal wound.

In the dog we anaesthetized with propolis in combination with 0.25% novocaine at the ratio 1 : 1, the anaesthesia was obviously

quicker (after 1—2 minute) and it afflicted better conditions for operation.

When using the propolis extract according to the procedure described there were no changes of pulse, temperature, breathing or reflex excitability.

In the operated area we used no antiseptic or antibiotic in wounds (9).

In some sheep, 24 hours after anaesthesia, there appeared a swelling in the area where the anaesthesia was applied, which in 3—4 days after operation disappeared without having unfavourably influenced the curing of the wound. In dogs there was no similar reaction.

Data about the variations of the blood spectrum are presented in Table 1.

The operation damage was mostly manifest in the white cell formula in the animals with a big incision.

In the first three hours after the operation the number of white cells is increasing. This change is accompanied by neutrophylia, the moving of the nucleus to the left and by lympho- and monopenya. 10 days after operation, the formula begins to normalize.

3 hours after the operation the formula of the red cells indicates a replication of the number of erythrocites and an increase of the haemoglobine amount.

The normalizing of these data takes place at the same time with the leucocitary formulae. 3 hours after the operation, the VSH is high and then it remains unchanged.

Immediately after the operation one can see in dogs a decrease in the number of white cells accompanied by neutrophily, moving the nucleus to the left and also with a slight lymphopenia. The white cells formula comes back to the normal level during 10—12 days.

The changes of the erythrocites formula are manifest after the operation by the increased number of erythrocites and the increased amount of haemoglobine.

These data come back to the initial position after approximately two weeks since the operation.

After the operation the VSH is high. The experimental operations in sheep are determining a gradual decrease of the albumine, the change of the albumine-globuline rate and the increase of the globulins.

The modifications were manifest after 3 hours from the operation. Meanwhile, the increase of the globulines took place, being brought about especially by the alfa fraction.

After 5—6 days the α globulins are being normalized and a corresponding increase of γ globulins takes place.

The whole normalization of the serum albumins spectrum lasts about 10 days.

Table 1

Species of operated animal	Nr.	Method of anaesthesy and kind of operation	Erythrocytes			Hb%			White cells		
			before operation	after 3—4 hours	after 5 days / after 10 days	before operation	after 3—4 hours	after 5 days / after 10 days	before operation	after 3—4 hours	after 5 days / after 10 days
Sheep	3	local infiltration with hydroalcoholic propolis solution ; laparotomy	4050	5460	4610 4200	40.5	49	48 41	4800	7080	7600 5150
Sheep	3	5% novocaine local infiltration ; laparotomy	4400	5070	5700 4500	43	50	57 45	6100	7100	7600 6500
Sheep	4	propolis extract oral administration or intraperitoneal injection of the propolis watery extract 1 : 1	5900	4900	5000 4050	38	46 40	46 40	5150	6300	4600 5300
Dogs	2	local infiltration with propolis watery extract (1 : 1), gastrotomy	7250	7350		110	115		13150	11900	

Conclusions

1. The anaesthesy with hydroalcoholic propolis extract given locally and under the form of infiltrations is not weaker than the 5% novocaine solution anaesthesy.

2. The propolis is a very good anaesthetic in the area to be operated if injected under the form of watery extract 1 : 1.

3. The propolis alcoholic extract given in the proportion of 0.012 g/kilobody has a good anaesthetic effect.

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ANTIOXIDANT VALUE OF PROPOLIS

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The bactericidal values of propolis have been extensively dealt with (KIVALKINA, LAVIE, etc.), just as its chemical composition (VILLANUEVA, POPRAVKO). Recently communications have been published, concerning the antioxidant value of propolis. The investigations conducted by one of the authors of this paper and collab. (USHKALOVA, ALSUFIEVA et al.) have brought to the fore the efficiency of propolis in stabilizing the quality of frozen fish. A processing method has been perfected and is being introduced in production, which enables frozen fish to keep its quality 2—3 times longer.

The systematic study of the antioxidant values of propolis is interesting not only for certain branches of food industry and cosmetics, but also for medicine and biology ; unfortunately, such data are not available.

In this paper we give the results of our comparative study of the antioxidant values of propolis and of the extracts obtained by means of solvents with gradually increasing polarity.

We used propolis from which we removed both mechanical impurities and waxes because they have no antioxidant value. Purification was obtained as follows :

The product (from apiaries in Kazan district, Tiumen region) was crumbled, and its extract was obtained in a 1 : 10 hot ethyl alcohol solution. The extract was filtered for removing the mechanical impurities, and the filtrate was diluted with water — 70% concentration, and then cooled down to 5°C. By this operation, waxes were eliminated : the extracts filtered and washed with alcohol several times, until the filtrate had a negative reaction to flavonoid compounds (ultraviolet light). Then the filtrate was evaporated in vacuum and dried in vacuum, at a temperature not exceeding 50°C, until a constant weight was reached. A brown-coloured product with the appearance of resin was obtained, which we called the sum of the flavonoids because it contained all the flavonoid components of propolis.

For separating the active components from the total number of flavonoids we extracted by solvents with growing polarity : (1) 2 : 1 mixture of petroleum ether and benzene, (2) benzene, (3) carbon tetrachloride, (4) chloroform, and (5) acetone.

Solvents were removed from the extracts by distillation in vacuum until a constant weight was reached. The quantity of the dry matter obtained was determined, as well as the presence of flavonoids by paper chromatography in formic acid-acetic acid-water system — 3 : 3 : 2, with subsequent revealing of spots by luminescence under ultraviolet light, previously and after revealing in ammonium (See table).

CHARACTERISTICS OF PROPOLIS EXTRACTS

Propolis extracts	Efficiency (%)	Colour	Flavonoids identified by paper chromatography, formic acid-acetic acid-water 3 : 3 : 2 system
Sum of flavonoids	65	Dark brown	
Waxes	21	Dark brown	
Mechanical impurities	11	Dark brown	
Extraction :			
— with mixture of petroleum ether and benzene 2 : 1	1.70	Yellow	2 spots — Rf 0.96 ; 0.73
— with benzene	25.50	Light brown	4 spots — Rf 0.9 ; 0.83 ; 0.8 ; 0.78
— with carbon tetrachloride	9.10	Light brown	6 spots — Rf 0.96 ; 0.915; 0.90 ; 0.89 ; 0.78 ; 0.65
— with chloroform	46.80	Dark brown	6 spots — Rf 0.96 ; 0.90 ; 0.89 ; 0.80 ; 0.78 ; 0 65
— with acetone	14.30	Dark brown	5 spots — Rf 0.918; 0.840; 0.42 ; 0.30 ; 0.63
Solid residue	2.60	Sandy	

The antioxidant efficiency was determined by the weighing method — in oleic acid, and by accumulation of peroxides in melted pork fat. Previously, the oleic acid had been distilled twice in vacuum, and the pork fat was melted in vacuum at 60°C. The peroxide content was determined by iodometry.

The extracts were then incorporated into the substratum under the form of solutions of absolute alcohol. The quantity of alcohol was introduced into the control solution. The test was made at 40°C.

The kinetic curves indicating the increase in weight of the oleic acid in step with the sum of the flavonoids being added — in 0.2%, 0.1%, 0.05%, 0.02% concentration — against acid weight, indicates that the sum of the flavonoids in 0.2% concentration slows down the absorption of oxigen by the oleic acid.

When concentration is reduced to half, the effect declines substantially. Further reduction of concentration has almost no effect on the increase in weight of oleic acid. On the basis of the results of our investigations we determined the antioxidant efficiency as the relation between the induction period when the sum of the flavonoids was added and the induction period in the control.

The antioxidant efficiency of extracts was determined by the same method. It was found that the sum of the flavonoids in propolis, in 0.2% concentration is the most active of all extracts. With the reduction of concentration, the activity of the sum of the flavonoids slowed down, while the activity of the extracts in benzene and chloroform varies very little ; that is why, when in concentration of 0.1% or less, the sum of the flavonoids is less active than these extracts. The chain $2 > 3 > 4 >$ sum of the flavonoids $> 1 > 5$ is relevant. Extracts 1 and 5 — in all concentrations, are less active than the sum of the flavonoids. Extract 1 contains little phenolic substances, and therefore its activity is reduced : extract 5 is likely o contain substances — as for example terpenes, which induce oxidation.

The kinetic curves of accumulation of peroxides in melted pork fat with the sum of flavonoids being added — in concentration of 0.2% 0.1%, 0.05% and 0.02% indicate that oxidation of melted pork fat slows down in step with the decrease in concentration of the sum of flavonoids.

The antioxidant efficiency of the sum of the flavonoids in melted pork fat extracts — with 0.1% concentration of the sum of the flavonoids being added — is illustrated by the chain : sum of the flavonoids $> 4 > 5 > 2 > 1 > 3$. The chain of antioxidant activity in pork fat is different from that in oleic acid. This phenomenon is due to the different nature of substrata ; the capacity of oxidation of the oleic acid depends above all on the existence of a doublebond, and alteration of pork fat has a catalytic effect because of the great quantity of haemins. The activity of extracts by means of chloroform and acetone — which contain the most readily polarized substances, depends on both the antioxidant capacity and on the inactivation of metal ions.

Consequently, the activity of propolis and of its extracts depends on the nature of the substratum.

CONSIDERATIONS ON THE CHARACTERISTICS OF ALCOHOL PROPOLIS EXTRACT

(Synthesis of works issued between 1964—1972)

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The progress recorded about propolis therapeutics in recent years justifies the interest in knowing its immediate effect and also the belated one in order to respect the principle "primum non nocere" which means that propolis should not determine noxious responses.

In order to establish this claim our research was directed to the knowledge of the characteristics and responses determined in the human body.

Propolis is composed especially of flavonoids (3), (20), (24), which have a plant origin and this accounts for some of its characteristics. (5), (6), (7), (8), (9), (10), (11), (16), (17).

DEREVICI et al. have performed researches *in vivo* and *in vitro*.

DEREVICI et al. (8), in the first group of experiments found antibiotic values against some microbes : *E. coli* 026, *E. coli* 0 111, *S. sonei, Sh. dysenteriae, S. cholerae suis, E. typhi TO 901,* enterococcus *(Str. faecalis).*

It is worth mentioning the inactivity on Oxford streptococcus and *B. subtilis mesentericus* (8).

The inhibitory effect of propolis on the germination of some seeds was established, especially on hemp seed. The 1/20 dilution reduced half of the germination of this seed (8).

The reduction of the air-borne flora from the apiary may be attributed to the antibiotic effect of propolis. This fact was demonstrated by DEREVICI, Al. POPESCU and N. POPESCU (8).

8 colonies were developed in a Petri dish, from apiary material, 21 colonies from an orchard located at 200 m distance and 38 colonies from town material.

As concerns moulds, they developed on a Czapek medium, 7 colonies from the apiary and 18 from the orchard (8).

The same authors (7), (11) found the inactivity of propolis on bees fed pollen infested with *Aspergillus niger* and *Mucor mucedo*, mixed with honey.

In the bees' abdomens, the presence of sporangi and hyphas was observed.

A propolis inhibitory effect in the 1/3 concentration was manifest on Czapek medium in the case of moulds.

In another series of experiments, DEREVICI et al. (9), (11) have studied mice tolerance to propolis, establishing that the dose tolerated by a 20 g mouse was 1.05 g/kilobody.

According to the hystopathologic examination of the organs in animals which received *per os* a hydroalcohol propolis solution 1/40, DE-

REVICI et al. (11) found propolis pellets in the peripheric cells of a vessel in liver and in the internal vessels.

The use of a greater amount of propolis brings about fatty degeneration in liver cells. The kidneys presented a glomerular infiltrate and the spleen a hyperplasia of the white substance and also a megacariocitary response. The lungs presented an infiltrate of the alveola wall as compared to the control. The layer under the stomach mucous membrane had propolis pellets. From the above, it shows that propolis is diffused in the body even at the moment it enters and is found in pellets in the above-mentioned organs.

DEREVICI, SORU and DIMA (12) have studied the effect of the alcohol propolis extract *in vivo* on mice inoculated with Ehrlich ascitis.

The experiments were performed with a propolis mixture in different dilutions — 1/20, 1/25, 1/50, 1/100, 1/250 — with ascitic liquid kept for one hour at 37°C, before using them.

Ascitis in mice which survived was used in the dilution 1/25 in order to conduct the second experiment.

In order to make the third experiment liver and spleen triturate was necessary because of the absence of ascitis. The result of this inoculation was negative, which demonstrates that Ehrlich ascitis was sterilized because of contact with propolis. The effect of the first experiment was considered as follows : 1) according to the number of mice alive for each propolis concentration and according to the number of days they survived ; 2) according to the intensity of ascitis ; 3) according to the number of cells and to the vitality of ascitic cells, indicated by the reaction with vital colouring matter.

Another device of this experiment used propolis as a treatment during the evolution of ascitis in mice which had been inoculated with ascitis tumour nine days before.

The results revealed an inhibitory effect of propolis *in vivo*, on Ehrlich ascitis tumour.

DEREVICI and POPESCU (10) have done other research work *in vitro* as regards the effect of propolis on Ehrlich ascitis cells morphology by maintaining the mixture with contact at 37°C over different intervals.

Examination under phase contrast microscope : after having kept it 1 hour at 37°C revealed the vesicular disposition of cytoplasma and the circular disposition of nucleus chromatyne. The cells were covered with an amorphous material which agglomerated them.

Control ascitis had large size cells which were circular, with abundant cytoplasma, and agglomerated chromatine in the nucleus.

After 3 hours contact, the changes were much sharper defined : the amorphous material covered most of the cells in a corrugated stratum.

The cell structure was hardly visible as if it were rare cell spots which were in contrast with control ascitis cell (with an excentric visible nucleus).

Examination under direct light microscope: it was done after having coloured the smears according to the May-Grünwald-Giemsa method.

The control cells maintained at 37°C for one hour, presented karyokineses and a great affinity to ointment.

Cells mixed with propolis had sprayed chromatine, some of them were vacuolised and others presented mitoses.

A ten days contact showed distinct alteration : disappearance of cytoplasm, pycnotic nuclei (disposed in bulks or in chains). The control ascitis cells presented amitoses.

DEREVICI, ZALMANOVICI and ARDELEANU (13) studied the effect of propolis on leucogram elements, and the morphologic reactivity of guinea pigs' and rabbits' mesenteries which had received a hydroalcohol suspension with propolis. The experimental background was described by FILOTTI and DEREVICI (14).

The experiments on guinea pigs were performed on those animals which received propolis in subcutaneous daily injections for 12 days — 10 mg of active substance in 10 ml of water (1200 mg in all).

The leucogram of these animals was examined before and after propolis administration. From the slaughtered guinea pigs, 5 days after the experiment had come to an end, mesentery fragments were taken again according to the technique of BOQUET and DELAUNEY (2), by applying the DEREVICI and FILOTTI alternative.

In the experiments on rabbits immunized with the antigen *Salmonella paratyphi* AO in 5 intravenous sittings, the propolis hydroalcohol suspension was administered intraperitoneally reaching in all, 500 mg of active substance for the whole experiment.

The control animals received injections with the same quantity of alcohol in water, without propolis.

When examining the leucogram, the results indicated an increase in multinuclears.

Lymphocytes presented a decrease in number.

Monocytes were equilibrated in guinea pigs and rabbits.

After having slaughtered these animals parts from the guinea pigs' and rabbits' mesentery were taken again in order to do hystopathological examinations necessary to establish the stages when the clearance process of propolis entered the body in the form of hydroalcohol suspension.

It is a complex process in which propolis is phagocytated and carried from the place of inoculation into the body.

Successive hystologic changes reflected the metabolization of propolis to the final stage. The results obtained showed that propolis which contains mainly flavonoids, among other compounds and plant origin substances, does not bring about noxious effects on the body under our conditions and doses.

More thorough research is necessary concerning the later effect brought about in the body by this product prepared by bees from the resins of some trees.

It is very useful that one should investigate the potential antitumoral effect of propolis, results having already been given in this respect by DEREVICI et al. (12) and (10) in their research on Ehrlich tumour and also by some authors who participated in the Symposium on flavonoids held in Paris in 1969 (21).

The morphological aspects described by us resemble those obtained by PALMER et al. (19) on tumours by means of pressure — active substances (lauryl sodium sulphate, lauryl pyridine-chlorine).

The authors admit that by these morphological changes the substances might have an effect on the lypoproteins of the cell membrane.

BARRELT and HODES (1) believe that some metabolic changes come next, as : cholesterol elimination in the environment. SORU, DIMA, SZABADOS (22) have established breathing shut off in Ehrlich ascitic cells in contact with propolis.

The favourable effects of bioflavonoids from propolis suggest that this study should be continued by chemical investigators, medical doctors biologists etc.

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CONTRIBUTION TO THE STUDY OF PROPOLIS

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A. *"IN VITRO"* AND *"IN VIVO"* INVESTIGATIONS OF CHEMICAL AND PHYSICO-CHEMICAL PROPERTIES OF PROPOLIS

Foreword

During the last decades the therapy with propolis made much progress recording favourable results in a series of diseases, which was pointed out by the papers and communications delivered at the specialized symposia and congresses (5) (6) (7) (27) (28) (29) (30)*.

Some of them have already been included in the volume entitled "Propolis" issued by the APIMONDIA Publishing House in 1975 (26).

This volume is a valuable source of information comprising original works on different matters. In this respect we consider those on biology and clinical work completed with a documentation on preparations with propolis to be particularly useful.

The works by BRĂILEANU (3) (4), VELESCU, MARIN (32) open up new possibilities of standardizing different ways of using propolis as a "medicine" according to sanitary legislation in this country.

We also endeavoured to make a contribution towards enlarging knowledge of properties of propolis and of its main components, the flavonoids. With that aim in view we conducted investigations in co-operation with different authors, biologists and chemists and the results are given in this synthesis paper, which includes 2 parts.

1. *Chemical and physico-chemical investigations*

a) A first series of research in co-operation with SORU (10) aimed at determining some chemical features of propolis. The use of different solvents allowed to point out the existence in propolis of some chloroform soluble fractions. After vaporization of chloroform a residue of 9.36 to 100 g is obtained.

The chloroform extracts dissolve lipids, a brown component, as well as other components thought to be responsible for the amber odour specific to flavonoids. This residue is subject to methanol extraction, which after evaporation leaves also a residue of 4.73 g%. The 3rd extraction gives the smallest amount of residue, namely 3.16 g to 100.

* Those interested in references may obtain them from the Editorial Office or by applying to the author.

All these 3 residues put together amount to 98.25 g to 100 g propolis. The total nitrogen is 0.400 g to 100 g of native propolis. The acid hydrolysis reveals by ascendent Watman paper chromatography 8 aminoacids : 1) serine, 2) glycine, 3) aspartic acid, 4) glutamic acid, 5) alanine, 6) tryptophan, 7) phenylalanine, 8) leucine. It should be noted that 3 of them are essential aminoacids.

The colorimetric techniques applied to warm water extraction of propolis to determine flavonoids gave positive results.

b) Other chemical and physio-chemical investigations of propolis were conducted by BOERU and DEREVICI (1) by using the chloroform residue obtained in the previous work or crude propolis. The chloroformic residue treated with acetone and then with methanol and subject to circular chromatography shows 11 spots.

Crude propolis used up by different organic solvents and subject to polyacryl amide gel electrophoresis shows 9 fractions.

As positive chemical reactions there were pointed out the colorimetric ones for flavonoids and that with orcinol.

c) GROZA, BLOOS, DEREVICI (23) using the japanese apparatus for determining automatically the amino-acids in a closed circuit, identified on propolis acid hydrolysate 18 amino-acids, among which 7 essential amino-acids; the 8th, the tryptophan, was not identified by this apparatus.

Crude propolis used in our experiments came from different regions of Romania. We quote some authors who contributed to the investigations destined to determine flavonoids in native plants. TAMAS (31) studied bilberries (*Vaccinium myrtillus* and *Vaccinium vitis ideae*). MIHELE (25) determined flavonoids in *Hieracium auranticum*, CONSTANTINESCU et al. (8) determined those in *Inula uliginosa*.

CRISTEA et al. (9) pointed out 17 amino-acids in the alcohol extract of *Tilea argentea*. As is known alcohol helps extract flavonoids.

In conclusion, these works point to the complex structure of propolis, but the identification of the reported fractions requires further investigations.

2. *"In vitro" and "in vivo" investigations*

DEREVICI, POPESCU and POPESCU (13) (14) (17) used in these researches a propolis hydro-alcoholic suspension (1 : 10) with ethyl alcohol extract. This is obtained by macerating crumbled particles of crude propolis in proportion of 25 g to 100 g alcohol 85°. The preparation is kept in brown tight jars at room temperature for 5 days and stirred several times a day. After this interval it is allowed to settle, when particles and coarse material insoluble in alcohol fall to the bottom. The resulting liquid is brown and clear. It will be kept in brown tight bottles in a cool dark place. To establish the amount of active substance we must determine the residue by a constant weight to 100 ml extract, which varies with the samples from 8 to 10 g to 100 ml extract. In this way we succeed in knowing the dose of active substance used to prepare the hydroalcoholic emulsion which assumes a uniform milk-like aspect (physiological salt should not be used because it makes the preparation precipitate).

As a rule two-thirds of alcoholic extract are evaporated and the controls of the experiments are given the corresponding concentration of ethyl-alcohol.

DEREVICI, POPESCU and POPESCU (17) (18) established that the dose of active substance tolerated by mice, guinea pigs, rabbits is 1.05 g/body weight.

Bees fed on honey mixed with 20% hydro-alcoholic emulsion show symptoms of paralysis followed by death (fig. 1).

Fig. 1 — Smear from haemolymph of bee fed on honey and propolis. Haematocytes contain propolis granules of a certain refringency. Col. May-Grünwald × 1250. According to DEREVICI et al. (18)

Fig. 1 a — Sporangium of hyphae in the abdomen of bees. Col. H. pheric × 500. According to DEREVICI et al. (18)

The corresponding dose of alcohol is not toxic to control bees. Propolis showed antibiotic properties towards some species of collon bacilli, dysentery bacilli and typhus bacilli, but not towards Oxford staphylococcus and *S. subtilis mesentericus* (13) (14).

To titre the antibiotic power of the preparation, these authors suggest the use of limit dilution technique in case of *Pasteurella avis*.

Bees infested by feeding them on honey maize pollen infected with *Aspergillus niger* and *Mucor mucedo* are not protected by propolis (15) (16). Although granular propolis does exist in the macronucleocytes of the haemolymph of bees, sporangiums and hyphae appear in their abdomen (17) (18) (fig. 1 a).

Volatile substances originating from the hive containing bees have an inhibitory action on the aeroflora in the neighbourhood of the hive.

The number of colonies cultivated on gelose or Czapek media for moulds is rather small in comparison with those obtained at 200 m from the hive, in the orchard or in the town (13) (14).

The hydro-alcoholic propolis emulsion has an inhibitory action also on the germination of hemp seeds (13) (14) and on the influenza virus when cultivated on egg embrion in course of development (24). These results differ from the control for which similar amounts of diluted alcohol are used.

DEREVICI and POPESCU (19) studied the effect of propolis on tumorous cells of Ehrlich ascites (fig. 2). When in direct contact with

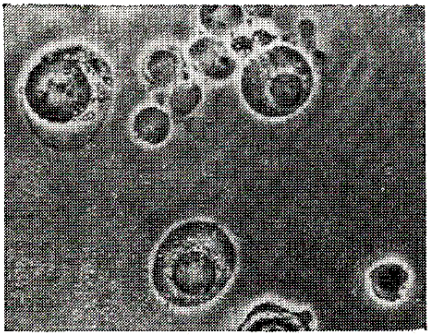

Fig. 2 — Ehrlich tumorous cells. Some of them are of large size and round, and their more or less eccentric nucleus shows agglomerated chromatine ; the abundant cytoplasm shows mobile refringent formations ; other smaller cells show less cytoplasm and their nucleus occupies nearly the whole cell. Phase contrast microscope examination of non fixed material × 500. According to DEREVICI et al. (19)

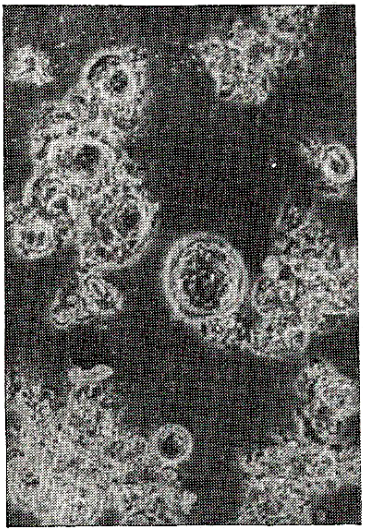

Fig. 3 — Ascites cells, after 3 hour contact ; they are covered by amorphous material ; rare cell shadows in the field. Examination under phase contrast microscope × 500. According to DEREVICI et al. (19)

the hydro-alcoholic emulsion of propolis extract the tumorous cells change progressively with the duration of contact. After 1 hour at 37°C the cytoplasm assumes a vesicular aspect round the altered nucleus. The cells are covered by an amorphous material which hides their structure and makes them agglomerate. The morphological changes are still more marked after 3 hour contact, when the amorphous material covers most of cells assuming a rough aspect. which was established under a phase contrast microscope (fig. 3) or on material fixed and coloured by the May-Grünwald-Giemsa method.

By *"in vivo"* experiments on mice DEREVICI, SORU and DIMA (20) determined the inhibiting action of the hydro-alcoholic propolis emulsion on the vitality of Ehrlich tumorous cells. The serial passages of ascitic liquid coming from animals surviving the last passage remain sterile whereas the tinctorial affinity of inoculated cells decreases.

Continuing the investigations of the reactivity of body to propolis, we conducted several experiments with guinea pigs and rabbits. In cooperation with ZALMANOVICI and ARDELEANU (21) we studied its effect under different experimental conditions as described by FILOTTI (22). In the experiments with guinea pigs, the animals are injected daily for 12 days with 100 mg active substance diluted in 10 ml distilled water (1200 mg in all). In the experiments with rabbits immunized intravenously with *Salmonella paratifi* AO antigen the animals are also administered propolis hydro-alcoholic emulsion in 5 sittings intraperitoneally (500 mg active substance in all). The control animals are injected with the same amount of alcohol diluted in distilled water without propolis. In these experiments neither the stimulation of the immunity processes of the increase in the antiparatyphus antibodies AO nor the increase in the alexic titre were obtained.

The examination of the leucogram of the experimental animals shows an increase in the neutrophile polynuclears with the contribution of guinea pigs being prevalent. The number of lymphocytes decreases and that of monocytes is balanced.

After slaughtering the animals samples of mesentery are taken by applying the BOQUET and DELAUNEY technique (2). Once fixed and coloured the sample is examined under the microscope in order to determine the stages of transport from the inoculation seat and the propolis "clearance" process.

The images show that particles of the hydro-alcoholic colloidal emulsion are drawn by capillary endothelial cells and appear under the form of granules surrounded by a halo. After several stages they are crumbled in fine granules, the macrophages interfere in their metabolism, and digestive, intracytoplasmatic vacuoles appear (fig. 4). A work by DEREVICI, ARDELEANU and ZALMANOVICI (11) deals with the results of the histologic examination of organs of the same animals. We mention that the sections examined showed no propolis granules whereas they were found in the organs of mice that received propolis *per os* (18). These animals showed also a slight fatty degeneration, which

Fig. 4 — Cytoplasm with vacuoles containing propolis granules. Col. H.E. × 1250. According to DEREVICI et al. (11)

suggests a different specific reactivity and points out the importance of the way of administration.

During the histological examination a particular attention was given to the determination of possible morphological changes of teratogenic nature. The results showed no such aspects. As a matter of fact we obtained the same results when examining in co-operation with ATHANASIU, PETRESCU, STOIAN (12) the sections from the organs of cubs of hamster inoculated when 48 hours old with propolis hydro-alcoholic emulsion. The examination was performed 6 months after inoculation during which interval no cutaneous macroscopic lesions appeared in the cubs of hamster under observation over all this period.

B. EXPERIMENTS AND INFERENCES ON THE ACTION MECHANISM OF FLAVONOID COMPONENTS OF PROPOLIS

This part of the work deals with an important component of propolis namely the group of flavonoids which is known from the investigations conducted by chemists.

The development of centres of investigation and documentation of pharmaceutic industries opened up the possibility of making rigorous scientific experimental studies towards determining these products. Their empirical therapeutical use preceded the study of their structure as well as the reactions caused in the body.

Several works in co-operation with chemists determined the complex structure of propolis and its flavonoid components. It contains glucides, protides and lipids, which could explain the possibility of its integration with physiological metabolisms.

According to SWAIN (34) the flavonoids are present in the vegetables in proportion of 36%.

Here are some aspects of the flavonoid component existing in propolis also, which were studied by different authors (6).

VILLANUEVA et al. (38) succeeded in separating some flavonoids and their works show a parallelism of the spectrographic curve with synthetic flavonoids (fig. 5). The analysis of the fatty acids of propolis in comparison with those of resins from poplar buds made by HEINEN and LINSKENS (19) shows a semblance between the chromatographic curves. This points out one of the most important sources used by bees to manufacture this natural produce that acts on the terminal blood circulation.

The interest in the group of flavonoids was aroused by the works of SZENT GYÖRGY (35) (36) who obtained good results with the total citrus juice applied to scurvy cases, particularly in haemorrhages. Besi-

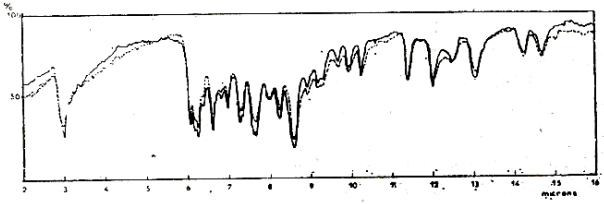

Fig. 5 — Spectrographic curves of galangine separated from propolis. The continuous line belongs to galangine; the discontinuous one — to synthetic galangine. According to VILLANUEVA et al. (38)

des vitamin C it contains another factor which works synergically and which we called permeability vitamin (P) or *citrine*.

The term of vitamin was refuted by some authors and replaced with that of bioflavonoids or flavonoid derivates. According to PARROT and CANU (28) and on the basis of the works by GAZAVE (29) the term factor C_2 is considered more adequate thus pointing out the synergism of its action in presence of vitamin C (9) (10) (11) (12).

During their action on the resistance of capillaries the two fractions from citrine give a biphase curve, the 1st of the adrenalinic type which lasts 24 hours and the 2nd which occurs 72 hours after (and lasts for several days) (11).

Properties of flavonoids

The studies of these substances dwell on the specific activity that decreases the fragility and permeability of capillaries. Simultaneous actions of numerous factors interfere in the function of the terminal vascular network (23), which is represented by lymphatic arteriol, venous, capillaries spread all over the body.

The anatomic structure of capillaries considered until not long ago as being simple (24) is in fact complex as far as its morphology and function are concerned. According to new data, the membrane of cell is not a static formation, but a lipidoprotean envelope showing a continuous dynamism. It plays not only a delimitation part but it also interferes actively in maintaining the intercellular ratios and in holding the balance in the exchange of the interstitial fluid.

The complexity of the processes occurring in the capillaries results from the unitary aspects of veins, lymphatic capillaries and nervous ends in the capillary layer.

Elements differing in structure separate the blood plasma from the extracellular fluid. It results that the study of this factor, the flavonoids, cannot be limited to the vascular walls but it must also include the functional biosphere of microcircular layer where perivascular mastocytes and nerves form together with the small vessels a functional entity. Chemical mediators interfere in the capillary layer on which depends the normal peripheric haemo-dynamic condition, the filtration and motive power of capillaries. These represent the seat of exchange between blood and tissues, provide the nutritive elements and remove residues, functions which require a very accurate mechanism co-ordinated by the central nervous system.

Metabolizing flavonoids

We analyse the contribution by investigators to clearing up the metabolism of flavonoids introduced in the body in different ways.

The experimenters used different derivates of flavonoids but we are interested in the whole group irrespective of the produce used.

The question has been asked what happens to the flavonoids administered to man or animals. GRIFFITHS and BARROW (14) (16) (17) (18) consider that flavonoids undergo several clevages under the influence of the intestinal flora. The same phenolic compounds are obtained *"in vitro"* with a microbial flora alone.

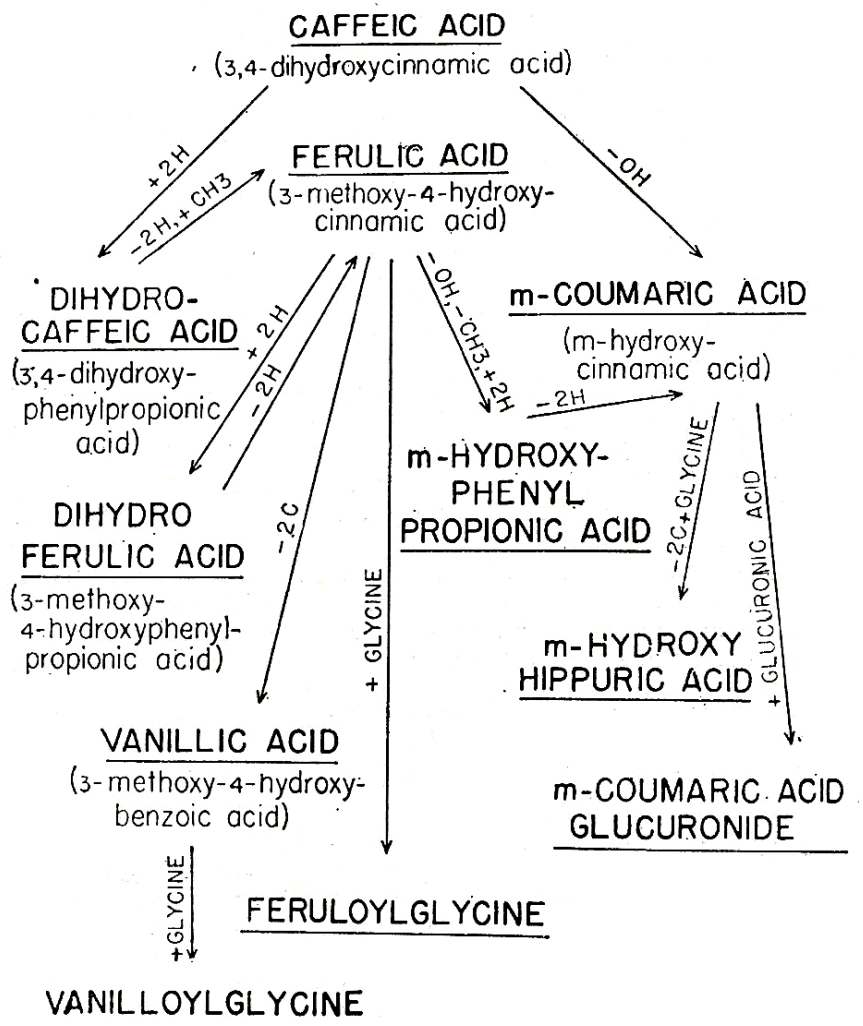

Fig. 6 — Urinal metabolites of the caffeic acid. According to BOOTH et al. (2), 1957

Experiments with animals raised under sterility conditions lead to similar conclusions (15). DAS and SOUTHY (5) also mention in their work the part the intestinal flora plays in the catabolism of flavonoids.

GRIFFITHS and BARROW use for their experiments a semi-synthetic product which contains 3 derivates of flavonoids differing in their structure as it results from their chromatographies made by MATAGNE (23). They also ascertain the appearance of metabolites in rats injected intraperitoneally. They reach the conclusion that hepatic enzymes occur during the clevage process and that the biliary tract is

a way serving to remove the resulted metabolites. In their experiments they used cannulas for exploring the biliary tract, which allowed to sample the bile. BOOTH et al. (2) (3) who started from the caffeic acid notice a large number of catabolism products which occur during the experiment and are removed together with the urine under the form of phenolic compounds (fig. 6).

Some differences are noticed depending upon the species of animals used and the administration of derivates of flavonoids. It is thought that it is possible to estimate the degree of catabolism by the amount of phenolic compounds eliminated by urine or feces and to establish an absorption index by time unit. The administration in the blood show higher figures for metabolites when flavonoids are administered parenterally.

During the metabolization the elimination is performed by biliary and urinary tracts.

The semi-synthetic "Venoruton" analysed by ZYMA contains derivates of rutoside called also Paroven whose solubility (25) differs and which is resistant to the gastric juice, but which is hydrolysed by the micro-organisms of the inferior intestinal flora. TAKACS et al (37) do not agree with the idea that intestinal flora interferes in the catabolism of flavonoids. They reach this conclusion on the basis of the results of the experiments on the liver separated from the general blood circulation and maintained by perfusion of a physiologic liquid containing flavonoids.

SIMPSON et al (30) studied the flavonoids metabolized by the microflora of rumen which they consider to be a good source of the microbial flora with which they obtain the degradation of flavonoids under anaerobic conditions. The results obtained with the rumen flora disappear by filtration through a Seitz filter.

BÖHM (4) quotes in his monograph on flavonoids several authors who studied the metabolism of the flavonic compounds. As to the duration of their appearance in the urine, some of them show 2 to 3 hours, some others several days. This difference is explainable because they used different ways of administration and also different animals : rabbits, guinea pigs, rats, cats, dogs.

Among the authors who studied the metabolization of flavonoids, CLARK et al. appreciate that only 1% of the administered dose is absorbed, the rest being removed unchanged.

STELZIG and RIBEIRO (33) find out that some flavonoids are removed by urine and some others by feces.

In contrast with the interference of flora in the clevage of flavonoids, already dealt with, MARCHELLI (22) reports the ability of *Aspergillus candidus* of synthetizing the flavonoids from preexisting elements in the medium. The contribution of such elements consisting of glucose, methionine, phenyl alanine occurs at the beginning of fermentation. The presence of flavonoids is pointed out with the help of the magnetic resonance spectrum.

Origin of troubles of circulatory diseases

Another category of investigations aimed at determining the functional and morphological incipient modifications favouring different apparently disparate circulatory diseases such as varicosis, arteriosclerosis, rheumatism, increased fragility and permeability of capillaries.

LASZT (21) shows that for a successful treatment it is necessary that these troubles should be detected in their incipient stage. He finds that varicosis is linked to troubles of the glucides metabolism, the amount of oxygen consumed by the fragments of the varicose vein is 3 times less and the production of lactic acid increases. The analysis of the structure of the venous wall of proteins, collagen, hexosamine reveals some modifications. Only elastine makes an exception.

NIEBES (27) studied the enzymes favouring the catabolism of mucopolysaccharides, namely : glucuronidasis, B acetylglucosaminidasis, acid phosphatase, arylsulphatase, hyaluronidasis and catepsin.

The activity of these enzymes save for catepsine in the varicose zone is increased. The author concludes that varicose alterations are caused by metabolism troubles of carbohydrates which inducing the instability of lysosome enzymes and releasing ferments bring about metabolism troubles of mucopolysaccharides and ultrastructural changes in collagen and elastine. Other authors also studied the changes in the conjunctive tissue specific to varicosis. ZWILLENBERG et al. (42) use cell cultures obtained by explanting the human varicose saphena, the bovine vein of the lower limb and the jugular vein.

By using different cultures they succeed in inducing alterations similar to those in the varicose vein. The constant occurrence of a collagen assuming a foamy aspect which appears in the medium tunic of the bovine vein 14 days after cultivation was called by them "Collagen associated structure" (CAS). In the culture medium appear N-acetylglucosaminidase and lactic acid and the activity of enzyme is lower in the hypoxic and anaerobic medium.

The same authors maintain that these processes are associated with active muscular cells of the vein, which is proved by the fact that CAS does not appear in jugular vein cultures, this vein being very poor in muscular cells.

To obtain such effects the authors dwell on the pH rigorous conditions which must be observed. The flavonoids added to the culture medium inhibit the occurrence of CAS considered to be an altered mucopolysaccharide.

In their further investigations of the conditions in inducing lesions similar to varicosis, ZWILLENBERG, FRIEDMAN et al. (41) incubate fragments of ligament conjunctive and obtain the same CAS formations under certain pH conditions and also the elevage of fibrils and helix. Comparative images appear in normal tissue and structures characteristic of varicosis. The authors consider the action of flavonoids on the ultrastructures studied when semisynthetic preparations "Venoruton"' are used to be of a minor effect.

To study the metabolism in the wall of artery, FILIPOVIC (7) used a culture of segments of aorta in a medium including triglycerids ;

a greater oxygen consumption under the influence of added flavonoids was noticed. No important influence on the lactate production was noticed.

MATAGNE and HAMOIR (23) studied the glycolitic enzymes of varicose and normal veins maintained in sarcoplasmic extract of varicose saphena, psoas and cardiac muscle.

The starch gel electrophoresis reveals some differences in proteins and in the distribution of lactate dehydrogenase which tends more to an anaerobic metabolism. But they found no differences in the activity of glycolitic enzymes. An increase in the activity of lysosomic enzymes is reported by MIRKOVIC (26) as a result of his experiments on thrombosis experimentally induced in dogs.

ZEMPLENY (40) considers that the preferential localization of lesions of the central atherosclerosis is induced by some haemodynamic and haemorheologic factors in association with local hypoxia. This favours the release of histamine, increases the permeability and disruption of mastocyte perivascular cells. The release of histamine favours the depositing of colloidal particles of lipids on the vascular wall on the background of the modified permeability. The flavonoids have the property of influencing this factor hence they are useful in preventing atherosclerosis.

KATO and GÖZSY (20) pointed out the intensifying effect of the activity of adrenalin.

PARROT and GAZAVE (29) and GAZAVE (9) (10) (12) (13) called this activating fraction Vitamin C_2. This factor vies with ortomethyl transferase enzime which interferes in the first stage of metabolization of adrenalin (15). The flavonoids vie with the metabolic process of adrenalin, hamper it temporarily and extend the duration of the action. The flavonoids seem to be in this process a factor that saves and protects adrenalin. It is a synergic action in association with the ascorbic acid, which is in its turn a competitive ortodyphenol of the enzyme catecolortodyphenoltransferase. Other opinions on the action mechanism of flavonoids admit that the influence on adrenalin occurs indirectly by stimulating hypophisis and by the action of corticotrope hormones ACTH on surrenal glands.

It is also stated that flavonoids act directly on the wall of capillaries inducing the vasoconstriction of precapillaries. The closing of precapillary sphincters could be induced by an antienzymatic action namely an inhibition of phosphorilation of ADP in ATP, which is absolutely necessary to the muscle relaxation.

As to the mechanism of action of flavonoids we consider it useful to close this account by quoting the interpretation by two competent chemists. According to SORU (31) the compounds with the function of vitamin P work as a reversible oxidoreducing system in synergism with the ascorbic-dehydroascorbic acid.

SZENT GYÖRGYI (35) (36) states that the oxidation process would be associated with the ascorbic-peroxidase thus establishing a possible correlated action between vitamins P and C.

In some deficiency diseases, these products are not effective when separately used, only their concomitant administration having a synergic action.

Following the interference of vitamin P at the level of the reversible oxidoreducing system the adrenalin-adrenochrome would reduce the oxidation and destroying speed of adrenalin which plays a certain part in the capillary resistance.

The vitamin P as an oxidoreducing system would play a part in the mechanism of the transfer of hydrogen. By this action vitamin P interferes in different respiratory metabolic processes of cells, in the metabolism of glucides and in the protein, ion and water one.

According to BESANGER-BEAUQUESNE (1) we can only make conjectures about the function of the group of flavonoids. He also states that these play a part in the oxidoreducing phenomena in plants. SZENT GYÖRGYI admits that when flavonoids interfere in the respiration of cells in plants provided with peroxidases, they are hydrogen carriers and their fate is linked to the ascorbic acid.

Flavonoids are a go-between in the oxidation of ascorbic acid. Oxidases act directly whereas peroxidases decompose peroxide produced by direct oxidations, turn flavons into quinones which in their turn oxidate the ascorbic acid for it to take again phenolic form and thus the oxidoreducing cycle is resumed.

The catalizing possibility is attributed to the phenolic nature of flavonoids. Their interference in the acceleration of the physiological systems in which the ascorbic acid also interferes would suggest that flavonoids play a coenzyme role. In our opinion, an aspect which must be taken into consideration is the chelating property of flavonoids ; they interfere competitively in different enzymatic processes thus inducing a series of important metabolisms described by GAZAVE et al. in the above mentioned works.

C. THERAPY WITH FLAVONOID COMPONENTS OF PROPOLIS AND TESTING RESULTS

This part of the work will not refer to the therapeutic results with propolis, which are extensively reported in the volume entitled "Propolis" edited by APIMONDIA in 1975, as well as in the specialized magazines.

We shall deal with : 1) the results obtained in apitherapy with the flavonoid component and 2) the methods of testing the apitherapeutic results.

The initiative of some pharmaceutic industries in providing some teams of research-workers with products with flavonoids for them to thoroughly study the various effects of such substances on the pathological states was very useful and the results obtained were presented and discussed at specialized symposia.

Some matters in dispute referred to the integration of flavonoids with the general metabolism but they were cleared up by biochemists who pointed out the presence of the metabolites of flavonoids in the

urine, bile and feces ; GILES and GUMMA (21) pointed out the presence in the serum of some voluntary patients of the flavonoids administered under the form of tablets. They used the technique applied by THIES and FISCHER (50).

Similarly by using auto-X-ray LAPARRA et al. (28) reported the stages of integration in the organs of a flavonoid marked by isotopes. DEREVICI et al. (18) described by histological examination the successive stages of the integration of propolis in the metabolism until the formation of enzymatic vacuoles.

To objectively estimate the properties of flavonoids the histologists use some experimental methods by which they induce circulatory disorders in animals which they treat with different derivates of flavonoids that will be dealt with as a general chemical group.

PRATESI et al. (43) induced regional ischemia by the ligature of carotid. After a certain interval he took samples of cerebral cortex in order to examine its ultrastructure. The control rabbit was also taken a cerebral cortex sample in order to examine its ultrastructure. Twelve hours after the ligature of carotid the capillary lumen became narrower and the cytoplasma thicker. The stroma showed numerous collagen fibrils. In the rabbit treated previously for 15 days with flavonoids and slaughtered 12 hours after the ligature of both carotids the basal membrane was of quasi-normal size and less opaque to electrons than in simple ischemia.

The rabbit treated with injections in doses of 100 mg/day showed a normal capillar ; during its halving the basal membrane comprised numerous pericytes. The cell limits and the endoplasmatic organellae were of normal aspect.

CETTA et al. (11) treat with some flavonoids lesions similar to those of varix induced by aminoacetonitriles. He finds the influence on collagen and the endothelium of small vessels is good. Fig. 7 gives the average variation of soluble collagen (in NaCl 0,45 M) in the aorta of rabbit lathyrised and protected by flavonoids. The white column represents the controls and the black one gives the value of soluble collagen raised in animals lathyrised with acetonitriles. The hatched column gives the decrease in soluble collagen in animals treated concurrently with flavonoids (fig. 7).

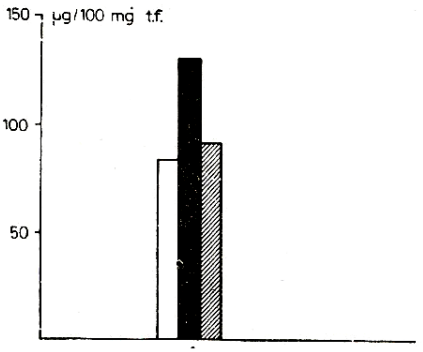

Fig. 7 — Average variation of soluble collagen in the aorta of rabbit treated with flavonoids. According to CETTA et al. (11)

To study the effect of flavonoids HAMMERSEN (25) induces oedema in rats by using dextran. The flavonoids are administered either *per os* 7 days or intravenously 1 hour before dextran. The slaughtering of the animals is preceded by their injecting with coal in order to mark the lesions. The controls do not receive dextran. Samples are taken 1 and 4 hours after dextran.

The previous treatment with flavonoids determines a smaller number of excrescences and reduces the thickness of basal membrane.

Nevertheless the conjunctive tissue does not become completely normal.

To study the influence of flavonoids on the deficiency diseases it was necessary to develop new methods.

The capillary fragility and the increased permeability foccussed the attention of clinicians.

The fragility of capillaries can be pointed out by inducing a positive pressure which suppresses temporarily the blood circulation in veins. To this end they use the clamp of the blood pressure apparatus. The bruises appearing in a certain surface are counted and the appearance time is determined. Another technical procedure consists in obtaining a depression by using a little rubber cup. On the market there is a Parrot angiosterometer (40).

WILD and FASEL (57) apply these techniques to point out chronique venous insufficiencies accompanied by hepatic cirrhosis. They determine the capillary resistance of the rectal mucous membrane and the time required by appearance of bruises, after which they compare the results obtained before and after the treatment with flavonoids (a Zyma preparation called Venoruton).

After a week of treatment one can notice the extension of the time required by the appearance of bruises.

PARROT and CANU (40) determine the increase in the capillary resistance by the number of bruises occured after the treatment with different flavonoids.

The permeability of capillaries may be determined by the Landis test which consists in inducing the venous stasis with the help of the clamp of the sphygmometer and in comparing the results obtained in each arm. The amount of liquid is determined : 1) The overflowing one of capillaries ; 2) the haematocrit, and 3) the amount of proteins in the serum.

The clamp of the sphygmometer is applied for 30 minutes to one arm only. This procedure was applied by WISMER (58) to different groups of patients before and after the treatment for 4 to 8 days with flavonoids and he found a decreased volume of filtration and overflown proteins.

The permeability of capillary vessels can also be appreciated by the test of diffusing a colouring matter (as a rule Evans tripan blue) in a region previously irritated by chloroform. This technique is used by LOISELLEUR et al. (34) by applying two injections of flavonoid (Flacitran), at 24 hours interval and the colouring matter after 6 hours of

rest. He finds that the flavonoid prepared from citrine associated with magnesium ions delays the diffusion.

PARIS and MOURY (39) modified this technique. To determine more objectively the appearance and intensity of the colouring of tegument they use a photoelectric cell to which they adapt a galvanometer for registering from time to time the variation of the diffusion of colouring matter, thus obtaining favourable effects with flavonoids.

GABOR (20) points out the inflammatory role of flavonoids and studies their action on the permeability of capillaries by the test of diffusing the Evans colouring matter (intravenously injected) in the irritated region.

The slaughtered rats are skinned, the fat is removed from the skin and the colouring matter is extracted by pyridine. The amount of colouring matter is appreciated photometrically on a given surface. It has been ascertained that the permeability decreases following the delayed diffusion in the animals treated with flavonoids.

Another method of studying the permeability of capillaries was suggested by CALNAN (8). To appreciate the degree of filtration towards the interstitial liquid a fenestrated capsule is introduced subcutaneously fitted with a device for sampling the interstitial liquid at different intervals. In the sampled liquid there are determined the values of certain constants which enable the appreciation of the permeability of capillaries.

For the study of capillary fragility and permeability in the ocular affections there is a very effective means of control by using fluoresceinography. The method allows to take the photography of fluorescence occurring on retinal arterioles.

The inoculation of the fluoresceine solution requires a special rather complicated preparation as it results from the work by TSCHOPP (53). Photographies are taken at different intervals. Colour films can also be used to study the eye-ground. The comparison of photographies allows to appreciate the development of diabetic retinopathy following the administration of flavonoids and simultaneously of the group receiving placebo. The criterion for appreciation is the presence of micro-aneurisms and overflown fluoresceine.

The photographies are used to draw schemes on tracing paper of arterioles, veins, overflown fluoresceine and micro-aneurisms. The schemes are distributed to several physicians to estimate them separately, after which their opinions are compared.

During each period of treatment the patients receive 400 mg HR 3 times in 24 hours for 2 months; placebo is also administered for 2 months after which the cycle with HR is repeated.

The authors conclude that this procedure is more accurate and allows to determine the effect of the treatment. No new haemorrhages occur but the existing ones remain unchanged. A diminution of permeability caused by fluoresceine is also noticed. But the effect is only maintained over the treatment period.

To estimate the effectiveness of the treatment of venous circulatory diseases, CAUWENBERGE et al. (10) use a derivate of rutoside, Veno-

ruton Zyma. Among other methods of inducing the inflammation, they use the subcutaneous implantation of a cotton wool tampon, which brings about an abscess. The animals of a group are treated with flavonoids in various doses which also brings about an abscess.

Other research-workers such as LUND et al (35) induce experimentally oedema in rats by compressing the basis of tail. The intensity of oedema is estimated by the volume of water removed in the calibrated cylinder annexed to this. These authors (35) estimate the neutralizing effect of some flavonoids by using groups of animals treated and non treated with HR. To induce oedemas they also used injections of adrenalin-ergotamin performed at the basis of tail, 12 hours after they administered HR for 5 days at 12 hours intervals.

THULESIUS and GJÖRES (52) studied the viscosity of blood in patients showing a chronic venous insufficiency. The viscosity is determined before and after the treatment in the cubital blood as well as in that sampled from the lower limbs.

No important differences between the treated groups and the controls appear. This fact determines the authors to exclude the contribution of viscosity to the venous circulatory disorders, which is contradictory to the conclusions drawn up by LOISELLEUR et al. by their experiments with the diffusion of colouring matters in animals.

The importance of a collateral blood circulation following ischemic accidents caused by the interruption of blood circulation in the coronary arteries and in the arteries of lower limbs was studied by BRKIC and LASZT (7). They developed an apparatus which allows to obtain a mecanogram. This is the response of the nerve corresponding to the muscle in which the collateral blood circulation was induced. In the experiments on the heart an electrocardiogram is used. The treatment of the collateral blood circulation with Venoruton gave significant results.

Another technique of studying the action of flavonoids was developed by SCHLEBUSCH and KERN (49). These authors started from the resemblance between the chemical structure of some flavonoids and that of polyphenols used in dressing (tannins) which would suggest that flavonoids could also act as stabilizers of collagen. It is known that fibrils of collagen are contracted and decrease in size, thus evolving heat and energy which can be measured with the help of an apparatus. According to these authors flavonoids would act as a dressing agent similar to the action of gold salts used in treating polyarthroses. The action of flavonoids is proportional to concentration.

Therapy with flavonoids

The therapy with flavonoids was applied to patients showing symptoms of deficiency diseases caused by their absence, such as scurvy, increased fragility and permeability, circulatory disorders in the lower limbs, varix, atherosclerosis, oedema, burns.

To determine the deficiencies one or more methods of those described about are used. As a rule one works with controls that are administered as placebo, an inert product having the same form as that studied. The pharmaceutic industry placed at the disposal of research-

workers preparations that had to be objectively tested and for that reason the experimenter himself did not know the nature of the preparation. There were used semisynthetic products, derivates of the rutoside extracted from the yellow acacia flower and other preparations containing citrine sometimes associated with metal ions. Other extracts of forest fruits (bilberry, black currant etc.), or chest-nuts etc. were also used.

On the basis of the activity of clinicians and the objective laboratory tests valuable conclusions are drawn on the therapeutic effects of a tested product.

Most investigators point out the absence of toxicity of flavonoids and their high tolerance when administered either in large doses or for a longer time. It is this property that prompted many investigators to study the therapeutic effects of flavonoids among whom we mention DEMURE (14) who worked with Difrarel extracted from bilberries; CLEMENT (13) (14) who worked with citroflavonoid products; PREROVSKI (44), KAPPERT (24), RAZGOVA (45), CAUWENBERGE (9), McEVANS (36), FILIPPI (19), LECOMBE and CAUWENBERGE (32) use semisynthetic products of rutoside called HR (Venoruton) (55).

The latter (32) reported some aminoreleasing derivates of rutoside marked Z 4000 but only on rats in which they noticed a sudden decrease in the blood pressure followed by tachyphilaxis.

The authors consider that these phenomena do not occur in man for whom other derivates of the rutoside are used.

LAGRUE et al. (25) studied the circulatory disorders occurring in atherosclerosis, high blood pressure (27) which they treated with visible improvement in 2/3 of cases. The *per os* treatment (100 mg × 3 in 24 hours) lasts 3 weeks and after an interruption of other 3 weeks, it is resumed. They used Esculoside Ld (25) called Folescutol (26). In another series of experiments ROSE obtained an improvement in the subjective symptomatology of varicosis and postphlebitis symptoms. That is why he considers the flavonoids to be useful in the therapy of circulatory troubles of lower limbs.

LECOQ recommends the use of flavonoids in purple and other haemorrhagic diseases (scurvy) in which initial ecchimoses are frequent, in haematuria, nephrites, sclerosis and high blood pressure, menstrual disorders, hepatic imbalance. GÖTZ (22) reports a decrease in cholesterol and satisfactory results with Concentrin (a preparation from chestnuts) applied in varix, varicose ulcerations, thrombophlebites. PARIS and MOURY (39) who check the effectiveness of flavonoids in permeability troubles by the diffusion test of the colouring matter Evans blue and an electroreflectometer fitted with a galvanometer find that the increased permeability improves under the influence of flavonoids. This is also confirmed by ARTUSON (2) in burns and DEREVICI et al. (17) who treat the burns experimentally induced with a hydro-alcoholic propolis emulsion including flavonoids.

The documentation on the use of flavonoids in ophthalmology offers the possibility of stopping the development of affections in which retinopathies are prevalent.

WEGMAN (56) studied the role of enzymes in the processes of adaptation to intense light and using retinograms he found the flavonoids to be effective in such cases.

The investigations conducted by ALFIERI (1) of the rapid adaptation to dark showed that the absolute light threshold came down following the treatment with flavonoids. The authors explain the mechanism by conversion of the ineffective ultraviolet rays into visible radiations of longer wavelength. MASQUELIER et al. (37) and PERDRIEL (41) who studied the effect of citroflavonoids on the adaptation to dazzlement, agree to the results obtained by ALFIERI. NEUMAN (38) THOMAS and BORISAIN (51), ROMAN (46) apply the treatment with flavonoids to conjunctive angiopathies during prediabetes or diabetes. The preparation Difrarel is an extract of bilberries. PETERSON and HEATH (42) induce retinopaties experimentally by treating rats with iminodipropionitriles inhibited by flavonoids.

BAIDAN and OIȚA (3) treat ocular affections with solutions and ointments prepared with solvents containing organic amines of propolis. They obtain good results in burns of corneoconjunctivas and blepharoconjunctives.

BOLLIGER (5) used the flavonoids with good results under the form of spray in dermatology, treating pruritus, erythema, semi-closed varicose veins or ulcers leaving indurations. LECOQ suggested the use of flavonoids in purple and LECLERCQ (33) in psoriasis.

Similarly BALANGER and DAX obtained good results with flavonoids localizing tumours and RUDALI and JULIARD (48) noticed that flavonoids inhibited the diffusion of mammary tumours in mice caused by a continous pregnancy. RUDALI, DECHAUME and COUSTOU (47) obtain such results by using magnesium chelates of flavonoids. According to BÖHM (6) the chelation is a basic mechanism of action of flavonoids. But CLEMENTSON (12) (15) insists upon the synergism together with the action of ascorbic acid, the flavonoids being considered as factors that save this acid by which they interfere in the oxydoreduction phenomena.

THE PHYTOINHIBITORY CHARACTERISTICS OF SOME SUBSTANCES PRODUCED BY THE BEE COLONY (*APIS MELLIFICA* L.)

M. GONNET
FRANCE

Introduction

The are many examples which show that certain extracts from substances of plant or animal origin bring about phytoinhibitory phenomena. The inhibitors of the germination or growth of different plant organs were discovered, as : roots of *Helianthemum, A. phyllanthes, Papaver, Thymus, Rosmarinus* etc. (I. BECKER, GUYOT 1951 ; DELENIL 1951) ; leaves, stems and buds of *Xanthium, Centaurea, Allium, Lycopsis, Pelea, Salix* etc. (KONIS 1947 ; FLETCHER, RENNEY 1963 ; LANGE, KAN-

ZOV 1965 ; GARESTIER et al. 1966 ; KEFELI 1966) ; fruits and seeds of *Lycopersicum, Vicia, Oryza* etc. (KONIS 1940 ; PEYRONEL, VARGA 1966). On the other hand, certain extracts of some animal secretions also present inhibitory activity : the steroles, the hormones and the diastases proved to be active for plants (LUSTIG and WACHTEL 1939 ; BOITEAU and RATSIMAMANGA 1958 ; ČIŽKOVÁ and ULRYCHOVÁ 1964 ; NITSCH and NITSCH 1965 ; MILCU 1966 ; RATSIMAMANGA 1966 ; MAC LAREN and BRADFUTE 1966). Human spittle contains an inhibitor of plant germination (YARDENI 1948). Finally, some authors have studied the inhibitory effect of the antibiotics on the germination and growth of plants (BARTON, MACNAB 1954 ; DUQUESNOIS 1955 ; BRIAN et al. 1965).

In insects there is also phytoinhibitory effect due to some secretions. According to PAVAN (1958) the inhibitory effects of iridomyrmecine (extracted from *Iridomyrmex humilis)* on the species *Lupinus albus* and *Allium cepa* was obvious. Also the cantaridine secreted by *Lytha vesicatoria,* and pederine produced by *Paederus fucipes.*

According to LEMAY (1947) bee venom controls seed germination. PAVAN (1958) isolated royal jelly from a bee secretion which is strongly inhibitory of the growth of *Lupinus albus.* Our first results were obtained by using rice *(Oryza sativa)* cultivated on absorbent cotton wool as substratum. In the seeds' water, water extracts were introduced from bee products. We worked with propolis, pollen, honey, wax and royal jelly extracts.

Honey and propolis brought about a total inhibition of rice germination.

DEREVICI et al. (1964) remarked an inhibition of the germination in *Cannabis sativa,* propolis alcohol extracts entering in the composition of the culture media used. These authors insisted especially on the inhibitory power of the different propolis grades which had been used. We studied the effect of propolis especially on the seeds of lettuce *(Lactuca sativa).* The inhibition is manifest depending on the concentration of propolis used by slowing down or even stopping plant growth. This last work is concerned with the inhibition of plant growth and not with the inhibition of germination as the former authors had considered.

Material and Methods

A. The nature and extraction of some substances extracted from the hive.

The extracts we tested were prepared as follows :

— *Water propolis extract* (P1), 80 g of propolis were extracted with boiled distilled water. The extract was taken, concentrated on a water bath and filtered ; 1 cm^3 from this extract contains 95 mg of dry matter ;

— *Propolis alcohol extract* (P2). 80 g of propolis are extracted with alcohol (1 hour with withdrawal). The extract was filtered the first time hot, then in Büchner device, the second time cold after the precipitation of the waxes. The alcohol evaporated. The residue was retaken in distilled water, cooled to 0°C, centrifuged and filtered. 1 cm^3 of filtered product contains 50 mg of dry matter.

— *Alcohol extract from pollen pellets collected by bees* (P8). 20 g of pollen from pollen pellets collected from a colony of bees (hive nr. 225) were ground and extracted with alcohol cold. After filtration the alcohol was evaporated. The residue mixed with water is cooled at 0°C, centrifuged, then filtered. 1 cm^3 active solution contains 118 mg of dry matter.

— *Bee bread alcohol extract* (P3). 20 g of bee bread from the combs of the same hive (hive nr. 225) were extracted and treated in the way described above. 1 cm^3 of extract contains 148 mg of dry matter.

— *Honey extract* (C8). The acid saponifiable fraction of an esterified honey extract. The way of obtaining this fraction will be the object of a minute description on another occasion. 1 cm^3 of watery solution contains 3.3 mg of dry substance.

— *Wax acetono-alcohol extract* (c). 10 g of old wax were dissolved in acetone, under heat. The acetone partially evaporates. It is extracted and diluted with water, decantated in the refrigerator (°C) for 24 hours. It is centrifuged and filtered. 1 cm^3 of filtered product contains 154 mg of dry matter.

— *Bee alcohol extract* (A). 50 g of taken bees (colony nr. 225) are macerated in alcohol for 24 hours, then extracted for 1 hour in withdrawal. After a first filtration into a Büchner apparatus, the filtered alcohol evaporated and the residue dissolved again with water. The extract put in the refrigerator for 24 hours is centrifuged, and then filtered again. 1 cm^3 of this solution contains 270 mg of dry matter.

The phythoinhibitory effect of all these extracts on the growth of *Lactuca sativa* was tested. As a consequence, we maintained the material and control methods which satisfied us in our first trials (GONNET 1966).

B. Biological samples for the species *Lactuca sativa*.

This method already used has to be described in more detail taking into account some improvements.

We used as culture medium 20% gelosis (in distilled water) in which we added either the solution to be studied, or water as control.

These mixtures were at once put on to Petri dishes (10 cm in diameter). The concentration of the extract used on the substratum is expressed in mg (dry matter) at 1 cm^3 of medium. The study of a concentration of this extract is performed with the help of 3 boxes, in each of them 100 seeds being sown. The seed used is Batavia lettuce (Blonde de Paris). The growth takes place in the drying closet at a tem-

perature of 25°C ; the relative humidity of the room is 80%. 16 hours after insemination, the plants were exposed to light for 30 minutes. This action is repeated 5 times during the whole period of the experiment (after 16, 24, 40, 48 and 64 hours).

The source of light has 2 fluorescent lamps of 40 watts each (Philips blanc brillant). The lighting received for each plate is 1 500 luxes.

Records are taken 70 hours after insemination. From each plate, 20 siblings are taken again as follows :

In order to take seeds at random, a cardboard disk is placed above the plate having a diameter of 10 cm. On this disk 5 circles are drawn — 2 cm in diameter placed at an equal distance (one central circle and another 4 circles around). This operation is performed by transparency in the interiour of each circle. The results are expressed in the length of hypocotyls and of the little roots (measured with a binocular magnifying glass) according to the ratios :

$$\frac{\text{(Length of hypocot.) } I^n}{\text{(Length of hypocot.) T}}$$

$$\frac{\text{(Length of roots) } I^n}{\text{(Length of roots) T}}$$

in which I^n represents the dilution in substratum of an inhibitory extract I and T is the control.

Results Obtained

All extracts obtained brought about (in different concentrations) a stop or a slowing of the plants growth. This inhibition is the strongest at the root level. The picture (fig. 1) illustrates the compared activity of all these substances.

Discussion and Conclusions

All substances extracted from the hive possess, in different degrees, the ability to inhibit or slow the growth of lettuce plants. We mention especially the interest which an acid saponified fraction extracted from honey presents. This slightly inhibits the development of the plantlets. Propolis extracts are equally remarkable.

The inhibitory fraction, present in propolis, is easier to extract with alcohol than with water.

The bees and wax extracts have a rather weak effect. Finally, the pollen deposited in combs has a reduced activity and the fresh pellets of pollen are even less active.

Thus the phytoinhibitory power of the fraction extracted from the body is weak as compared to the activity of the products harvested by the bee. Naturally, this leads us to the supposition that the inhibitory substances of plant growth which are present in honey and propolis are, at least to a great extent, of plant origin. We can assert that the deposited pollen, already mixed with spittle secretions and pressed into the cells of the combs by bees, is more active than the pellets of pollen in which hardly any alteration begins.

The harvesting of these categories of pollen was from the same hive and over the same period. The two types of samples taken again were thus comparable qualitatively.

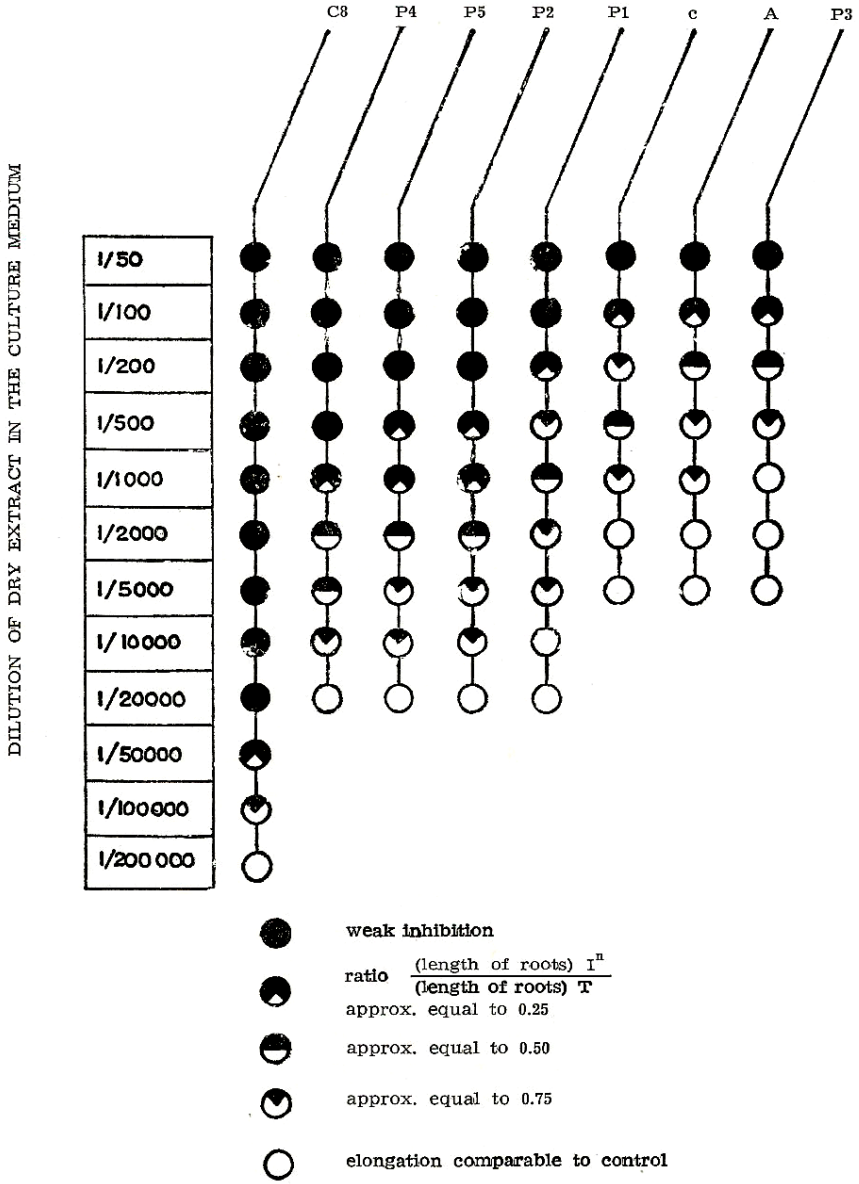

Fig. 1 — The phytoinhibitory effect of some substances extracted from the bee colony on the roots of *Lactuca sativa*

On the other hand, MAURIZIO (1960) found that certain salivary secretion of the bee, included in fresh pollen (*Papaver betula* — pollen harvested by hand) hinder germination and the pollen tubes' growth. Bees' alcohol extracts gave him the same results.

So, the double origin and the manifold substances with phyto-inhibitory character are obvious in the products of a colony of bees.

This phenomenon is not at all surprising because, as we could see, animals as well as plants may accumulate substances which induce plant growth in certain tissues.

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IV. PROPOLIS EFFECTS ON DIFFERENT BIOLOGICAL PROCESSES

INFLUENCE OF SOME EXTRACTS OF PROPOLIS ON MITOSIS IN *ALLIUM CEPA* L. MERISTEMS

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Visible remissions in some cases of malignant tumours and leukaemiae reported by some authors as a result of the administration of propolis under different forms determined us to study the cytologic mechanism of action of propolis on some vegetal meristems whose cells were genetically unbalanced just as with cancerous cells.

This paper gives the preliminary results of our experiments in this direction with radicular meristems of *Allium cepa* L. in which we watched the development of mitosis, its frequency and normal structure.

Material and method

Rooted bulbs of *Allium cepa* L. with numerous roots 2 to 3 cm long were summarily examined for the normal mitosis and passed on a "Proposept" solution (propolis alcoholic extract diluted in water by adding ethylene diamine) or on a mixed solution of saturated griseofulvin (about 10 mg/l water) plus 0.02% Proposept. At 24 hours intervals 5 small roots were collected and fixed in a Battaglia fixative, coloured according to Feulgen and treated by a "squash" method. These stabilized preparations were used to watch the development of mitosis by observing its quality and counting the division of cells in order to determine the frequency of division and the mitosis condition.

1st experiment. Rooted bulbs were allowed to continue their development on a 1% "Proposept" water solution. This concentration is high enough for the possible cytostatic effect suggested by the remissions of cancerous tumours and leukaemia to appear rapidly and to the full and our observations confirmed it. The frequency of mitosis in the meristems of roots diminished gradually and disappeared nearly completely 48 hours after roots had contacted the 0.1% propolis solution. But

what is worth mentioning is the fact that this mitodepressive effect which is obvious enough is accompanied by neither structural modifications in the mitosis, nor a derangement of the achromatic spindle (mitoclasia), nor the appearance of some chromosomal aberration whatsoever, nor side effects, i.e. bridges, fragments, micronuclei, included in the term of chromatoclasia.

This seems to be particularly important because we think that an oncolitic (or at least oncostatic) substance having to be mitodepressive must not engender informative unbalances either under the form of chromosomal or genomic mutations (aberrations in the 1st case, polyploidy, aneuploidy in the 2nd). It is known that characteristic features of many types of cancer consist just of such modifications in the number of chromosomes, which results in the first place in an anarchic and invading development, this being the proof of systematic non-integration of malignant cells with the organism.

In this stage we interrupted the influence of propolis and allowed bulbs to keep on developing in tap water. Subsequent observations on meristems showed that the first mitoses reappeared merely 24 hours after interruption of the influence of propolis and they showed a perfectly normal structure. Meristems treated with propolis reach a normal frequency of mitosis 72 hours after cessation of its influence, and by this time roots resume their normal development.

Thus a first conclusion on the influence of propolis on the mitosis of normal meristem cells has already been set forth and we shall only draw it up again :

— *propolis contains mitodepressive principles* that do not *engender mitotic anomalies or chromosome aberrations, having a strong but reversible action after interruption of its influence.*

We could only expect that the mitodepressive action of an oncolitic or oncostatic substance should be at least equal or stronger on some genetically deranged cells (such as cancerous ones) and if possible mitosis should no more appear in them.

2nd experiment. In our opinion it was interesting to administer "Proposept" to some deranged cells in point of integrity of the genetic apparatus even though they did not become malignant. We used griseofulvin as a deranging agent, which, as reported by the specialized literature and as we also know from our own results (not yet published), strongly and entirely deranges mitosis inducing pseudo-metaphases, pseudo-anaphases and pseudo-telephases of colchic type, but also — very often — multipolar anaphases which result in plurinucleate cells with non-equivalent nuclei showing a deficiency in the genic content. At a concentration of 10 mg/l (saturated water solution) with which we worked, after 24 hours of action we found a slightly increased frequency of mitosis (an increased mitotic index) but all the mitotic figures were modified. After 4 days of action mitosis disappears, but nearly all meristematic cells are plurinucleate and polyploid. Probably the plurinucleate state prevents the entering into mitosis, whence a mitodepression.

The influence of propolis added to the experimental assay in proportion of only 0.02% (as mixed water solution) is very significant the more so as its concentration is 5 times less than in the 1st experiment.

The mitodepressive action (of propolis) in the assay with griseofulvin + propolis in comparison with that treated with griseofulvin alone is very strong particularly on cells with deranged mitosis which disappear rapidly; only mitoses with slight derangements (meristemokineses) persist for a longer time as slightly dispersed metaphases and anaphases, with retardants etc.

Perhaps propolis mixed with griseofulvin blocks the preparations for mitosis particularly in cells whose ciphre was deranged (genetically deranged cells) and for this reason strongly deranged mitoses appear no longer as they do when griseofulvin is administered alone.

Under such conditions mitoses in meristems disappear completely after 3 days but due to propolis and its mitodepressive effect no plurinucleate cells appear. This could lead us to the conclusion that the mitodepressive effect of propolis is much stronger on abnormal mitosis and genetically deranged cells than on normal cells, which — as already mentioned — would mean to ask too much of an oncolitic or at least oncostatic substance.

It should also be noted that after passing onion bulbs from griseofulvin and propolis solution to water, mitosis — either normal or modified — does not appear even after 5 to 6 days in radicular meristems treated in this way. This makes us assume that mitodepressive principles of propolis act more strongly and for a longer time on the meristem cells showing a derangement or a tendency to genetical derangement. This could create a prolificness advance in favour of normal cells, which would favour regeneration of a normal meristematic tissue in place of that affected by a deranging factor.

Discussions

If we refer to the action of mitodepressive principles of propolis on the cells whose ciphre was deranged (genetically deranged) by griseofulvin or by getting malignant, the cases of remissions of cancerous tumours and leukaemia mentioned at the beginning of this paper could be explained by a similar action.

A tissue never gets malignant entirely; it will always contain some normal cells but their activity will be affected or even repressed by malignant cells. By repressing preferably and for a long time malignant cells, propolis would favour — even though indirectly — the activity of normal cells, which would help the body reestablish its normal condition. Thus the development of malignant cells could be slowed down or even stopped if not jugulated.

Even if we do not admit the oncolitic or oncostatic action of mitodepressive principles of propolis, the experiments described above suggest a more general principle for the therapeutic research on cancer, namely:

In our opinion the treatment of cancer does not require strong cytostatics which would necessarily affect normal cells in continuous division (epithelial, haematopoietic cells etc.) in the human body and which could possibly result in their getting malignant; slight cytostatics and cytotoxics would be sufficient. These would affect little, or not at all, normal cells which have a strong cellular homeostasis by their inte-

gration with the normal system of the body, but they would affect strongly (until their destruction) malignant cells non integrated into the system of the body and which for this reason have a slight physiological homeostasis thus being easier to destroy and eradicate.

We assume that extracts of propolis posses such qualities although many laboratory and clinical experiments are still necessary, which will prove their oncostatic or oncolitic effects, if any.

Conclusions

1. Propolis contains mitodepressive principles but they do not engender mitotic anomalies or chromosomal aberrations and their action is reversible in the case of normal meristematic cells.

2. When administered together with a deranging factor of mitosis such as griseofulvin their mitodepressive action is particularly strong on genetically deranged cells, and in particular it seems to be irreversible.

3. Further experiments are still necessary to determine the relation between some remissions of cancerous tumours and leukaemia in men as a result of the administration of propolis and the cytological effects of this bee product pointed out by our experiments with vegetal radicular meristems.

PROPOLIS IMPACT ON THE IMMUNOGENESIS IN THE CASE OF IMMUNISATION WITH TETANIC ANATOXIN

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Propolis has drawn attention to itself lately more and more as being a nonspecific stimulator of the immunologic reaction. In the department of microbiology of the Kazan Institute of veterinary medicine they established the favourable impact of propolis on immunogenesis by its administration concomitantly with the salmonellic corpuscular and noncorpuscular antigens (V. P. KIVALKINA and V. A. BALALYKINA, 1969 ; A. I. BALALYKINA, 1968 ; I. I. TETEREV, 1969 ; V. P. KIVALKINA, I. V. PIONTKOVSKI, 1969, and others).

The present work follows the study of the effect of propolis on the immunologic rates by its simultaneous administration with tetanic anatoxin. Two series of experiments were conducted.

In the first series they studied the impact of propolis on the creation of antitoxin and the activity of the complement by hyper-immunization of rabbits with sipped purified tetanic anatoxin. The experiment was performed on 20 rabbits — Chinchilla breed — which weighed 2—3 kg each and were 6 months old. The animals were divided into 4 groups.

The first and third group were hyperimmunized with sipped anatoxin and the second and third group with the same anatoxin in combination with propolis. 5 injections with anatoxin were made, every

other 7 days. The rabbits from the first and the second group were injected with 20, 40, 80, 120 and 160 uf.

The rabbits from the third and the fourth group were injected first with the same levels and afterwards — with 160 and 240 fu. Thus, the rabbits from the first and the second group received a smaller total dose than those from the third and the fourth group.

Propolis was added to anatoxin under the form of alcohol extract (5 mg of dry matter to 1 ml antigen).

The blood serum was examined before hyperimmunization and then weekly, for a period of two months. The antitoxin amount was determined by the red cells passive agglutination reaction and by the neutralization reaction on white mice and the effect of the complement by titration in the haemolitic system.

By hyperimmunization of the rabbits with sipped tetanic anatoxin the appearance of the antibodies was noticed in blood two weeks after the first injection with anatoxin.

Then the antitoxin level increased gradually, reaching a maximum in the animals of all groups on the 42nd day. On the 49th day the antitoxin content from blood serum suddenly decreased and did not increase any longer ; however, a slight increase of the antibody titre was observed in the third group of animals.

The antitoxin content from blood serum in the rabbits immunized with a smaller total level of propolis-anatoxin was bigger than in those administered the same antigen levels without propolis. On the 42nd day the antitoxin amount in the rabbits of the second group which received a smaller propolis-anatoxin total amount, was 208 au. which means 2.2 times more than in the rabbits from the first group (control — 96 au.).

In the animals from the fourth group, immunized with a bigger level of propolis-anatoxin, a slight increase of the antitoxin level as compared to the level from the rabbits with the same anatoxin dose without propolis was observed. The maximum antibody content from the blood serum of the rabbits of these groups was almost identical — 121.6 and 120.8 au.

Thus, the increasing of the total anatoxin dose without propolis led to a more intense response of the organism. Propolis stimulated more the production of antibodies only in combination with a smaller total dose of anatoxin. In the case of a bigger total anatoxin dose, the addition of propolis did not produce such an effect.

During the whole experiment the titre of the complement in the animals which had received propolis-anatoxin was bigger than in the control groups.

Bigger titres of the complement were noticed in groups of immunized rabbits with a smaller propolis-anatoxin dose.

The data obtained demonstrate that the hyperimmunization of the rabbits with sipped propolis-tetanic anatoxin, stimulates the immunity non-specific factors and the specific ones, when using a smaller anatoxin total dose.

In the second series of experiments we studied propolis impact as adjuvant on the antitoxins production and the protection characteristics

of the sera, obtained in the hyperimmunization of rabbits with purified concentrated tetanic anatoxin. 18 rabbits — Chinchilla breed — 6 months old, were used in the experiment. They were injected 5 times, every other 7 days. The rabbits from the control group were hyperimmunized with purified concentrated tetanic anatoxin with doses of 20, 40, 80, 120 and 160 fu. The animals from the experimental group were injected with the same amount of anatoxin in the same periods, but not in combination with propolis.

In the experiments performed on 435 white mice weighing 16—18 g, one studied the prophilaxis characteristics of the blood sera in rabbits. To this purpose, the sera obtained from the control animals and from the experimental ones were mixed separately in equal quantities, thus obtaining a group serum which was administered to the white mice under the skin in the spinal area, in the dose of 0.5 ml. 15—25 mice were used each time. 24 hours after the injection with serum, the mice were infected by another injection with a certain amount of tetanic toxin (between 1 and 8 000 LD min.). Uninoculated but infected mice served as control. The clinical observations on mice lasted for 10 days.

In the case of mice hyperimmunization with concentrated anatoxin, the antibodies appeared in the blood in the 14th day after the first injection : in the mice immunized with propolis-anatoxin, their titre exceeded obviously the control level. (Table 1).

The anatoxin content from the blood serum increased gradually, reaching a maximum on the 35th day of observation.

At this date, the antitoxin amount in the rabbits from the experimental group was twice as much as in the control animals ($p<0.01$). Then the antibodies level decreased and on the 63rd day it was 0.48 ± 0.27 ua. in control, and 1.2 ± 0.19 ua. in the experimental groups. Thus, the amount of antitoxin from the blood serum was much bigger in the cases of propolis-anatoxin immunization than in the cases of anatoxin immunization without propolis.

If the injecting of mice with 30 MLD tetanic toxin led to the 100% death rate of the animals inoculated with mice blood serum from the control group during the appearance in their blood of antitoxin, the administration of the same toxin dose brought about the death of only 40% of the mice administered the blood serum of the experimental rabbits. On the first day of mice infection with 200 MLD of tetanic toxin, the death of 100% of the mice inoculated with the blood serum of the control animals and 15% of the mice inoculated with the experimental rabbits' serum was brought about.

Table 1

ANTITOXIN AMOUNT IN RABBIT'S BLOOD

Groups rabbits	Term of the research (days)							
	14	21	28	35	42	49	56	63
Control	0.88 ± 0.32	6.44 ± 0.8	14.5 ± 3.04	36.5 ± 7.5	16.0 ± 0	8.6 ± 2.05	1.37 ± 0.14	0.48 ± 0.27
Experimental	1.86 ± 0.38	10.66 ± 1.33	20.9 ± 3.7	74.6 ± 10.6	49.8 ± 5.6	18.0 ± 3.3	4.57 ± 0.65	1.20 ± 0.19
P	<0.05	<0.02	>0.2	<0.01	<0.001	<0.05	<0.001	<0.05

As the antitoxin accumulated in the rabbits' blood, the protective characteristics of the sera increased. The blood sera of the experimental animals prevented the mice from death to a greater extent than the blood sera of the rabbits from the control group.

On the 35th day of research, when one found the highest antitoxin level, the administration of the blood serum — 3 000 MLD tetanic toxin from the control animals to the inoculated mice led to 100% death rate.

Those inoculated with experimental mice blood serum had 100% death rate when the percentage of 60% IInd degree tetanus and 40% Ist degree tetanus appeared in the mice. On the 42nd day of research, when one observed the decrease of the antitoxin amount, the administration of experimental animals' blood serum (8 000 MLD toxin) in the inoculated mice led to the death of only 28% of the mice. The level of antitoxin in the research period in both groups was smaller than the initial one, although in this period the mice bore a 10 times bigger dose of toxin as compared to the initial period.

Thus, the research of the second series of experiments showed that as the antitoxin accumulated in the blood, the prophilaxis power of the sera increased. However the decrease of the antibody amount after reaching the maximum level did not lead to the decrease of the prophilaxis power of the sera. In all research periods the blood sera of the propolis-anatoxin immunized animals protected the mice to a greater extent that in the rabbits which received anatoxin without propolis.

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PLASMOCITARY RESPONSE IN WHITE MICE IMMUNIZED WITH AN ANTIGEN ASSOCIATED WITH PROPOLIS

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The purpose of our present study was to study the impact of propolis administered together with an antigen, on the plasmocitary response which constitutes one of the main proofs of the immunologic altering of the organism which is present soon after immunization.

Material and Method

The research was performed on 137 white mice. As antigen, a complex one was used (the glucido-lypoproteic complex) extracted from *S. enteritidis.*

The mice from the first group were immunized with antigen dissolved in saline solution ; the mice from the second group were administered the same antigen, emulsioned in a mixture of lanoline and vaseline ; the mice from the third group were administered antigen

emulsioned with supplement of saponyn. Those from the fourth group were given antigen emulsioned with a propolis stimulator. The antigen was injected only once under the skin in the right shank area. In the 3rd, 6th, 10th, 15th, 20th, 27th, 34th, 44th, 58th, 75th and 90th day after immunization, three mice from each group were narcotized with ether. They were opened and the following lymphatic ganglions were extracted: right popliteal, mesenteric, paraaortic, popliteals, left under-scapulars. On the blades prepared, the cells from the plasmocitary series were counted according to the method of M. P. POKROVSKAIA and L. S. KOGANOVA (1947). In parallelism with the study of cytologic alterations, the antibodies in homogenates were titred from the lymphatic organs and from the blood serum. From the lymphatic organs, homogenates were prepared and diluted with saline solution 1 : 10, 1 : 20. They were extracted for 24 hours at the temperature of 4°C, centrifuged, and the supernatant liquid underwent an agglutination reaction, the same as the blood serum — according to a current method.

According to previous researches on intact animals, one established that in 50 microscopic areas of the samples from lymphatic ganglions, 6—9 cells from the plasmocitary series were found.

Results of the Researches

The cytologic analysis on groups has shown that the immunologic response at the administration of the antigen is most intensely manifest and in very short time by increasing the number of cells from the plasmocitary series in the regional lymphatic ganglions.

In the white mice immunized with complex antigen dissolved in saline solution (group 1), one found a slight plasmoblastosis in the lymphatic regional ganglions, on the 3rd day after immunization.

The greatest number of cells from the plasmocitary series (91) was found on the 6th day.

Their level had a non-stable character.

In the peripheric lymphatic ganglions, similar cell alterations were observed, the difference lying only in a decreased intensity of the plasmocitary response.

In the regional ganglions homogenates, the antibodies were found on the 3rd—6th day after antigen administration. They reached the maximum number (1 : 1280) on the 15th day. In the lymphatic peripheric ganglions, the appearance of the antibodies began to be manifest only after the 15th day; the highest antibody titres were found later than in the regional ganglions (on the 34th and 75th day).

On the 3rd day of the experiment, 1 : 23 aglutinyns were titred in the blood serum. Their titre suddenly increased and reached the maximum value on the 44th day (1 : 3460).

In the animals immunized with complete antigen emulsioned in the mixture lonaline-vaseline (group II), an increase of the number of plasmatic cells was observed on the 3rd day of the experiment. The plasmoblasts were prevailing. The maximum number of cells (102) from the plasmocitary series was fixed on the 10th day after immunization.

Subsequently, their number came back to the initial value with an unimportant increase on the 58th day. In the peripheric lymphatic ganglions, the antibodies were identified on the 10th day and their maximum titres on the 58th day of the experiment.

The increase of the aglutinyns titres in the homogenates of lymphatic organs and of blood serum was parallel, and in the first period of observations the antibody titres in homogenates were superior to those from the blood serum. The greatest amount of aglutinyns (1 : 3733) from the blood serum was established on the 44th day.

In the regional ganglions of the white mice immunized with a complete antigen emulsioned in the mixture lanoline-vaseline with the addition of saponyne (group III), a slight increase of the number of plasmoblasts was found on the 3rd day. The maximum number of cells from the plasmocitary series (146) was fixed on the 6th day of observations. Subsequently, one found the decrease of their level up to the initial values, with a slight increase on the 44th and the 90th day. In the peripheric lymphatic ganglions the cells moved as those in the regional ganglions, but less intensely.

In the regional lymphatic ganglions homogenates, antibodies were revealed on the 3rd day after immunization. The maximum amount was observed on the 15th day — the titre 1 : 2560. The peripheric lymphatic organs gradually entered the process of the antibody genesis and their complete involvement was observed on the 15th day ; the 44th day they contained the greatest number of antibodies. The aglutinyns titre in the blood serum and in the homogenates increased at the same time with their quantitative prevailing, shortly after immunization in the homogenates.

In the blood serum, the maximum amount of agglutinyns at the titre 1 : 3733 was found on the 34th day the antibody titres decreased in the regional lymphatic ganglions homogenates.

In the animals, immunized with emulsioned antigen mixed with lanoline-vaseline and propolis addition (group IV), an increase in the number of plasmoblasts in the regional lymphatic ganglions was observed on the 3rd day after administration.

The maximum cell accumulation in the plasmocitary series (544) was observed on the 6th day of the experiment. The plasmatic cells were in groups of 5—20 in myelinic coordinates in the medullary area and very seldom in the wall of the lymphatic ganglion : immature plasmatic cells prevailed. On the 20th and 27th day their number was not worth mentioning. Mature plasmatic cells were prevailing.

The second increase of the cells from the plasmocitary series (117) was found on the 34th day of observation : towards the end of the experiment, the number was almost normal. The peripheric ganglions had a similar cell response. The maximum number of cells from the plasmocitary series was over normal.

In the regional ganglions homogenates, antibodies were detected on the 3rd day after immunization. Their level reached a maximum value on the 27th day at the titre 1 : 5120. The removed lymphatic ganglions

were included in the antibody genesis, on the 6th day. The maximum increase of the antibodies level was on the 27th day, the titre of the serum dilution being 1 : 13866. In these animals, the antibody titre in the regional lymphatic ganglions homogenates and in the blood serum increase parallel to the highest rates during the same periods. The plasmocitary series cells proliferation in the regional lymphatic ganglions was 3.7—6 times more intense in the white mice immunized with complete propolis antigen, than in the individuals from other groups.

Comparing the data of the morphologic and serologic research, we came to the conclusion that the increase of the number of plasmatic cells in the regional ganglions is preceding to the specific antibody accumulation. In the regional lymphatic ganglions' homogenates, the anti-

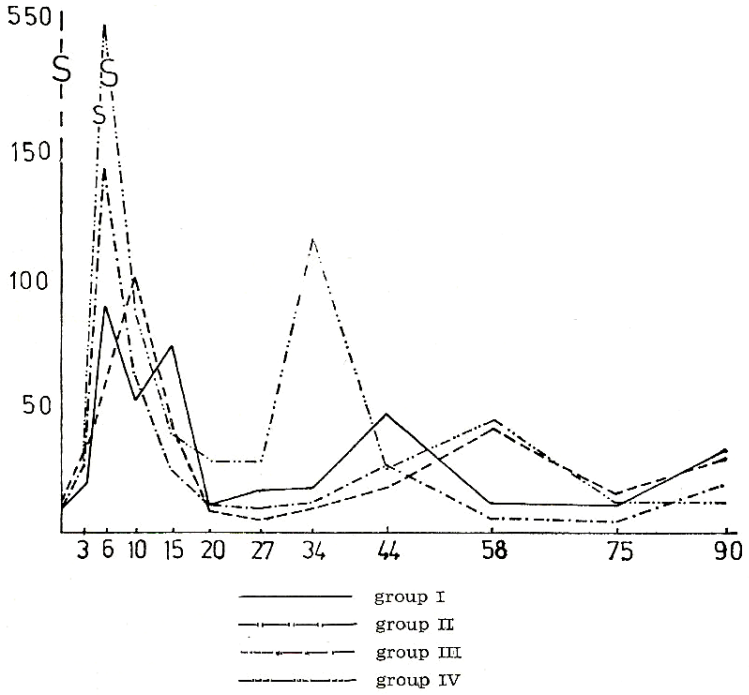

Fig. 1 — Dynamics of the cells from the plasmocitary series in the regional lymphatic ganglions of white mice

bodies were revealed sooner than in the blood serum and they were present in bigger titres. However, in the blood serum of the white mice innoculated with propolis emulsioned antigen, the antibodies existed during almost the whole experiment in bigger titres than in homogenates. This was due to the active involvment in the genesis of antibodies, not only of the regional lymphatic ganglions but also of the peripheric ones. The aglutinynes titres in the blood serum of the white mice immunized with propolis antigen were 3.7-4 times greater than those of

the animals inoculated with complete antigen combined with other adjuvants.

The same conclusions were also established from the comparison of the maximum titres of the antibodies from the regional ganglions' homogenates, among groups.

Thus the conclusion is that propolis, combined with antigens, stimulates the plasmocitary response and the appearance of antibodies in the regional and peripheric lymphatic organs.

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BALANCE-SHEET AND PROSPECTS OF THE STUDY OF PROPOLIS

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The curative properties of hive products have been known from time immemorial. Nevertheless their scientific study has begun recently and propolis has the youngest history. It was first studied under the Veterinary Institute of Kazan in 1947, which conducted investigations of its antimicrobial properties. By that time nothing was known of these properties of propolis and in point of its curative qualities it was known as a remedy for corns and that during the Boers' war a preparation called Propolisin containing propolis was used with much success in treating and curing the wounds.

The use of propolis by bees for covering the corpses of undesired visitors penetrating into the hive such as big insects, mice, a.s.o. suggested the existence in its composition of some antibacterial substances.

To confirm this hypothesis we made the following experiments: corpses of albino mice and pieces of fresh meat infected with different species of bacteria were covered with propolis. When we examined them to see what had happened we found that propolis had prevented them from putrefying. The corpses of mice and the pieces of meat maintained their initial aspect for a long time; then they began suffering certain changes. That was the underlying stimulus of the study of the antimicrobial properties of this bee product.

We studied the bacteriostatic properties of propolis and of different extracts of propolis by *in vitro* experiments by applying different methods. These investigations showed that propolis had a large antimicrobial spectrum i.e. it acted on both gram positive and gram negative species of bacteria. But it should be noted that not all of them are influenced to the same extent and on the other hand the antimicrobial action of propolis and of its extracts is not the same either.

Because the main natural components of propolis are resinous substances and these are not always identical in all species of plants one may assume a priori that the antimicrobial action of different

categories of propolis is not identical. The comparative study of a large number of samples of propolis coming from different zones of the country confirmed this hypothesis. Nevertheless we never encountered a sample of propolis without antimicrobial action.

The antimicrobial properties of propolis as well as the experience gained by folk medicine were the first premises of the preparations we used: paste of propolis, different propolis ointments, propolis emulsion in alcohol, water and milk. The experiments with albino mice, guinea pigs, rabbits, showed that propolis had no toxic action on them.

The therapeutical properties of propolis were studied on animals whose infection was induced experimentally and on naturally infected animals.

The first patients were animals suffering from necrobacillosis — bovines and horses. Necrobacillosis is a disease caused by the anaerobic bacillus of necrosis. This consists of necrosis of tissues affecting particularly the limbs, the buccal mucous membrane and the udder.

Besides necrobacillosis the preparations with propolis for external use — particularly as propolis ointments — turned out particularly effective in the treatment of infected wounds irrespective of their seats, but specially of those located in areolae and fissures of hoofs in bovines and of burns in dogs and calves.

The use with success of preparations with propolis in animals is a stimulus to use them in man too. The treatment with propolis was applied to out-patients as well as in clinics. The use of preparations with propolis for therapeutical purposes in man proved to be effective in different dermic affections such as eczemas especially in the chronic ones and in those of sucklings, in tricophytes and other dermatoses accompanied by strong pruritus, hydradenites, furunculosis, infected wounds, thermic and chemical burns, bruises, different gynecological affections such as erosion of cervix uteri, vaginitis a.s.o.

The foregoing refers to the external application of propolis. The component substances of propolis such as vegetal resins and their internal use seem to be incompatible at first sight from a pharmacological point of view. But facts are difficult to contest. The particular therapeutic effect as well as the non-toxic action of propolis on the body stimulated the experts to try its internal application too. Young animals used in agriculture are very sensitive to pulmonary and intestinal affections. Antibiotics and other drugs used to treat them have not always given the desired results.

Therefore it was necessary to develop other methods of treating them and other preparations such as propolis milk, a hydro-alcoholic emulsion, extract of propolis mixed with vegetal oil, vaseline a.s.o. The first patients on which these preparations were tested were diseased piglings, lambs and calves and the therapeutic effects exceeded all expectations. Based on the positive results obtained by external and internal use of preparations with propolis the Veterinary Institute of Kazan worked out recommendations for the application of propolis in veterinary surgery.

The gastro-intestinal and pulmonary affections in man are not less difficult, especially the ulcerous affections of the gastro-intestinal tract. There are works that developed an experimental pattern of ulcerous disease in laboratory animals and their treatment with success with preparations including propolis. It is likely that propolis should be applied together with other etiotrope means to treat pulmonary tuberculosis.

The therapeutic effect of new preparations is estimated in comparison with other therapeutical means which are widely used in practical medicine and propolis makes no exception. At the beginning the preparations with propolis were used when other preparations proved to be ineffective.

Every new preparation is given much attention even if its effects will be only partial where other therapeutical means gave no results. This was the case of propolis. Its application with success in similar cases resulted in the popularization of preparations with propolis. But the rule the sooner the better is true for propolis too. The effectiveness of propolis finds also expression in the fact that its application results as a rule in a more rapid cure. The pathological process is jugulated more rapidly than when other usual therapeutical means are applied, which is of paramount importance for both veterinary science and human medicine.

But what is the explanation of the high therapeutic effectiveness of propolis? What are the mechanisms of its antimicrobial and curative actions ? Up to the present we have no satisfactory answers to these questions. The biological activity of propolis is no doubt linked to its chemical composition but until not long ago very little was known of its components. It is only during the last years that this gap began to be filled. The application of modern methods of investigation allowed the separation and investigation of some individual substances in propolis. The study of the different sides of their biological activity will further allow to clarify the action mechanisms of propolis on micro and macro-organisms. Nevertheless one cannot appreciate the whole activity of propolis by considering only the activity of its components separately.

The preparations with propolis proved to have an antiinflamatory action. Thus to application of a propolis ointment results in a rapid and plentiful increase in the granulation tissues and desquamation of the necrotized zones. In patients treated with propolis the painful syndrome disappears. In animals this finds expression in the fact that immediately after application of a dressing with propolis they can set the diseased leg on the ground. In humans the signs of pruritus disappear. The disappearance of the sensation of pain and pruritus points to the anaesthetic action of propolis. This was also confirmed by experiments with rabbits, frogs and karakul sheep. According to data supplied by the authors who studied this matter the anaesthetic effect of propolis is stronger than that of cocaine and novocaine. The anaesthetic property plays a decisive role in the mechanism of therapeutic

action of propolis and this is a feature which qualifies propolis as a means of pathogenic therapy. Nevertheless the antimicrobial action also participates to a great extent in the therapeutical activity of propolis. This is particularly true for the local application of preparations with propolis and an evidence in this sense are the character of the modification in the microbial spectrum of wounds after application of a propolis ointment and also the fact that in mycotic affections of skin only concentrated preparations with propolis are effective.

It is probable that the internal application of propolis in cases of pulmonary affections should not have much importance. This is proved by the results of investigations of the influence of propolis on the quantitative composition of main intestinal flora which allow to draw the conclusion that internal application of propolis (hydro-alcoholic emulsion) does not result in disbacteriosis. How can we explain the therapeutic effect of preparations with propolis taken internally? The results of our investigations of the influence of propolis on the immunological indices give perhaps at least a partial answer to this question. Indeed our experiments with rabbits and guinea pigs showed that the internal administration of hydro-alcoholic emulsion brought about an increase in the immunizing factors both specific (genesis of antibodies) and nonspecific (complementary and phagocytic action, properdine amount). This points to the fact that the increase in the immunologic reaction of organism plays an important part in the therapeutic action of propolis particularly when taken internally.

The results of these researches underlay the tests on propolis as a nonspecific stimulant of immunogenesis when it was taken together with the antigen. The experiments were made with corpuscular and non-corpuscular salmonellic antigen and tetanic anatoxin on albino mice, rats, guinea pigs, rabbits and calves. These investigations showed that propolis inoculated into the body at the same time with the antigen stimulated the immunogenesis processes. The administration of antigen together with propolis results in a more rapid formation of tissues in the lymphoid organs particularly in the regional lymphatic ganglions. This finds expression in an intense hyperplasia of lymphatic tissue and a rapid formation of immuno-competent cells.

In this case it was noticed an increase in the complementary action of blood serum, phagocytic activity, synthesis of gamaglobulin and specific antibodies — agglutinin, precipitin, antitoxin.

Hyperimmune serums obtained as a response to the inoculation of salmonellic antigen and tetanic anatoxin together with propolis in mice had a more marked preventive action.

The results of these investigations point to propolis as an adjuvant substance and opens up the possibility of its use for increasing the effect of vaccins and serums. The quality of propolis as an adjuvant was studied in comparison with the adjuvant Freind which consists of a mixture of lanolin-vaseline with inactivated microbacteria of tuberculosis. Propolis not only is not inferior in terms of action to this adjuvant but exceeds it, engenders less reactions and does not cause allergies. In addition propolis has another important quality, namely bacteria do not become resistant to it.

Although we made numerous passages of *Staphylococcus aureus* and *Colon bacillus* in propolis media we did not succeed in changing their initial sensitiveness to this product. Likewise we did not succeed in separating resistant forms to propolis even in bacteria contained by propolis itself. The sporogenous species of bacteria separated from propolis do not develop in propolis media even under the conditions of an insignificant amount of extracted propolis they contain. This made us to study the antimicrobial action of antibiotics combined with propolis. The formation of antibiotic resistant bacteria is a serious disadvantage of antibiotics as well as of other antimicrobial populations, which decreases their therapeutic value. The antimicrobial action of antibiotics combined with propolis was studied in nutritive media and ointments. The experiments showed that propolis increased the antimicrobial action of penicillin, streptomycin, tetracycline, chloromycitine, neomycin, monomycin, oleandomycin, polymixin and ristomycin, extended their action but did not inhibit the *in vitro* appearance of antibiotic resistant forms. The results point to the possibility of using propolis in the antibiotic therapy which will allow to increase their therapeutic effectiveness. This combination has also the advantage that propolis belongs to the category of antibiotic substances of superior plants and animals, which are different from those of microbial origin; the former are less toxic and have a more marked physiological action whereas the latter have a lower microbial action. The combined application of antibiotics and propolis can increase the therapeutic effect in both external and internal administration.

The study of new preparations covers not only the positive effects but also the negative ones and the possibility of complications. It is on this basis that counter-indications in the use of propolis are prescribed.

Although there is a very rich clinical experience in using propolis, we do not know of negative effects of this product on the organism, save for some cases of intolerance or high sensitiveness to it materialized in local irritation, reddening and sometimes inflammation of skin. The high

sensitiveness to propolis occurs sometimes in apiarists in form of eczemas.

The foregoing shows that a very rich material concerning propolis has been accumulated, which confirms its biological activity. Further studies of propolis will open up large possibilities of applying it for therapeutic purposes in veterinary science and human medicine.

ALLERGY TO PROPOLIS

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Lately, in the waiting room of the allergology section of the Hospital nr. 1 — Moscow, a new category of sick person has appeared : those allergic to propolis.

Propolis has enjoyed for a number of years a good and widespread fame. Its application to certain diseases has a positive effect (usually for bronchial infections).

But there are some cases when propolis may bring about allergic responses of the body.

Here is such a case of allergy to propolis : the patient M appealed to the allergology section. She had put propolis on her forearm in order to treat a benign tumour. After 24 hours a strong itching appeared where the propolis had been applied and a sensation of smarting.

When the dressing was taken off the skin turned red and had a nettle rash. After 24 hours the rash had extended over the whole arm as a general rash. A hard oedema of the forearm, of the hand and of a part of the shoulder was noticed. The general condition worsened every hour. The patient suffered a sensation of faint cephalaea, sickness, and high fever (38°). The patient was hospitalized in the allergology section.

When making the anamnesis, it was found that the sister of the sick woman had a severe form of allergy to bee stings. Both of them were brought up next to an apiary and in childhood they had often been stung by bees.

Another case is that of bronchial asthma in a patient who had severe difficulty in breathing after inhaling propolis.

Another man, allergic to bee stings, had an anaphylactic shock, after being massaged with a 2% propolis alcohol infusion.

As a rule, allergic responses to propolis appear in the persons who have allergy to bee stings or to bees in general, as well as in other persons with allergies : bronchial asthma, eczema, rash etc.

It is not clear for the moment if the patients react to propolis or to the protein from the bee venom, which pollutes propolis. However, allergic persons are recommended to be specially careful when inhaling propolis especially when this is in strong concentrations (30—40%) because a long irritation of the mucuous membranes may render the person more sensitive to propolis (appearance of allergy).

EXPERIMENTAL AND CLINICAL RESULTS IN THE TREATMENT OF WOUNDS IN DOMESTIC ANIMALS BY LOCAL APPLICATION OF AN ALCOHOL PROPOLIS SOLUTION

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Even if in curing a wound one cannot reduce the healing only to a local treatment — other influential factors with biological components included, and also biochemical and ensimatic interfere. We studied the local effect of some antibiotics produced in our country (powder or spray) — Sulfopen powder, Framykoin powder, Antibiotic spray, Chronicin spray etc.

In this connection, very interesting were the works of ČIŽMÁRIK and MATEL, which besides the history of propolis and the study of its chemical composition, include a large review of the studies in the domain of the bacteriostatic and bactericide effects of propolis. Of late, ROMANOV (quoted by ČIŽMÁRIK 1969) determined the very intense reaction of propolis as a local analgesic, even if the chemical composition as an analgesic is not known. The importance of propolis for its use in medicine lies also in its biostimulatory effects on the protection power of the body. Thus, we decided to test the effects of the local application of propolis on the healing of different wounds in domestic animals. The preliminary experiments were performed on hospitalized animals and because the results were positive, we conducted some comparative experiments — by local application of propolis and of other medicines currently recommended for wounds for local treatment.

Material and Method

I. *Production of propolis alcohol solution*

First, we established the maximum solubility of propolis in 96% alcohol. To this purpose, native propolis, finely ground (including wax

and other impurities) was mixed with an electromagnetic mixer in 96% alcohol, for 24 hours. After the filtration of the propolis concentrated alcohol solution, the solution concentration was determined (on the basis of the known amount of alcohol — bulk and weight —, of rough propolis, of the width of the insoluble sediment and of the filtrate); this reached 11.36%.

Because the alcohol solution obtained was meant for the healing of wounds which are hardened by the 96% alcohol, it was necessary that we resort to a 60% alcohol solution which is good enough for disinfection. After the 60% propolis alcohol solution, a new precipitate which had to be again separated by filtration, appeared. According to the weight value of the different components, we appreciated that the saturated solution — 60% alcohol with propolis — contains 5% of the product. This solution was used for cicatrizing the wounds.

Since the preparation procedure described above proved to be more difficult and longer, we set up a chromatic scale — 5 test tubes with propolis solutions from 1% to 5%.

II. *Experimental part*

The experimental part of establishing the local effect of propolis on wounds was performed on 12 sheep, one and a half to two years old kept in the same winter stable with identical diet. After the preliminary preparation of the operation area with a local anaesthetic using procaine solution (1%), in each animal two wounds were cut (4×4 cm) whose depth affected the thickness of the derma.

The wounds were made symetrically on both sides of the spinal column, in the area of the hips, and at the level of the first lombar vertebra.

The experimental animals were divided into two groups, each consisting of 6 individuals. In both groups, the wounds from right side were treated by applying propolis on them and on the wounds from the left side we applied: on the first group Sulfopen powder (penicillin G, calcium 500.000 I.U., normal animal plasma mixed with dry mixture (3 g), benzosulfonamyde chlorhydrate + sulphanilamyde ad 20 g) and on the second group — Framykoin powder (neomicyne — 3.3 mg — Zn — bacitracyne — 250 u. to 1 g of powder). The results were observed on the first, second and fourth day after the appearance of the wound and every four days until the 24th day.

Before each treatment the wounds were cleaned.

Results and Considerations

When appraising the quickness of the wounds cicatrization we followed the evolution of the wound surfaces in cm^2. As a consequence of the suppurative process, on the first days after their incitation the wounds grew larger and the size was established only between the 8th and the 12th day.

During the first 12 days we could establish no essential differences in wound sizes.

Between the 12th and 16th day, a remarkable limitation of the wound surface was recorded, in all groups, more obviously in the wounds treated with propolis and a less remarkable one in those applied Framykoin powder and Sulfopen. The next period (the 20^{th}—24^{th} day), the process of epithelium growth continued as well as the limitation of wounds surfaces. This was more obvious in the wounds treated with propolis alcohol solution. From the statistics of the results it followed that in the 16^{th}, 20^{th} and 24^{th} day a statistically significant difference between the wounds treated with propolis and those treated with Sulfopen was recorded, as being advantageous.

Even if differences appeared between the evolution of the sizes of wounds treated with propolis and those applied Framykoin, they are not significant (table 1).

Table 1

MODIFICATIONS OF THE EXPERIMENTAL WOUNDS SURFACES (in cm^2) IN SHEEP, DURING THE CICATRIZATION PROCESS

Group		Days since wound appearance						Observations
		0	12	16	20	24	42	
Propolis p=12	X	16	10.53	3.84*	2.25	0.93*	0.02	* statistic meanings in the respective conditions $t_\alpha = 0.05$
	S			0.87	0.98	0.70		
Sulfopen p=6	X	16	10.68	5.32	3.76	2.19	0.42	
	S			1.62	1.66	1.54		
Framykoin p=6	X	16	10.60	4.37	2.66	1.12	0	
	S			1.00	0.92	0.69		

When controlling group treated with propolis, on the 42^{nd} day of experiment, out of the total of 12 wounds, 11 were fully covered with

epithelium ; in the group of those treated with Framykoin powder, out of 6 wounds, 5 were fully covered with epithelium. Out of 6 wounds treated with Sulfopen powder, 4 wounds were not completely covered with epithelium, even after an interval of 42 days.

In all three groups, during the first 2—4 days the wounds presented a dry aspect. In the group treated with propolis and with Framykoin (powder) the wounds began suppurating on the 6th and 8th day. This phenomenon was not observed in the case of the wounds treated with Sulfopen. Until the 12th day, the wounds filled with granulation tissue and in the group treated with propolis we observed a beginning of epithelium of 40%.

In the next period, a very intense growth process of epithelium was noticed in the group treated with propolis alcohol solution and with Framykoin powder. The wounds treated with Sulfopen even if they were dry and granulated were not covered with epithelium.

Thus we may assert that propolis alcohol solution has notable effects on the growth of epithelium on wounds. These effects were observed on horses, dogs and cattle.

The majority of the hospitalized patients were applied propolis only when the granulation of the wound was obvious.

Propolis led to a rapid drying of wounds. It also protected the granulation tissue.

Conclusions

1. We watched the effect of the alcohol propolis solution on the cicatrization of an experimental wound and we compared the results with the effect of Framykoin and Sulfopen powders in 12 sheep for experiment.

2. In the initial period, neither propolis nor Framykoin did obviously influence bactericidically the wound (which suppurated), but between the 12th and the 16th day one observed an intense epithelium growth (developing) process due to propolis.

3. We consider that the use of propolis alcohol solution in healing wounds, after having removed the wound infection, is necessary.

THE EFFECT OF PROPOLIS FRACTIONS ON SOME BIOLOGICAL SYSTEMS

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According to data obtained from literature it is certain that propolis shows multilateral biological activities. It is quite possible that the polyvalent effect of propolis originates from its chemical heterogeneity.

During our previous examinations of propolis we extracted by column chromatography 15 different propolis fractions. Five of the 15 fractions are chromatographically pure, and the others are a mixture of two or three components. The purity of some fractions was tested by thin-layered silica gel chromatography. All of the obtained propolis fractions are hard substances, with low melting points, except two which are very viscous fluids.

During our further research closer differentiation of propolis fractions is planned and in the initial phase first of all with biological tests. In this paper we shall present only the preliminary results of the examinations of the effect of three propolis fractions of various concentrations on mammalian cells in vitro.

The effect of B, D, and E fractions, each in concentrations of 0.5%, 1.5% and 2.5% added to a volumetrically reciprocal medium was studied. We used two groups of cell cultures as controls :

a. cell culture exposed to the effect of ethanol in concentrations of 0.5%, 1.5% and 2.5% and

b. cell culture without any effects of other compounds, except those in the medium.

In the experiments stable cell lines (fibrocytes) from rat tissue were tested. The cells were maintained in Parker-199 medium with 20% calf serum and antibiotics. The cells were exposed to the effect of fractions and solutions for 12 hours.

Two criteria for the estimation of propolis fractions on the cells culture were established :

1. Determination of mitotic index, and
2. Occurrence of chromosomal aberrations.

At the end of the experiments the preparations were treated with the standard techniques, using also colcemide, hypotonic solution and fixatives (alcohol and acetic acid, 3 : 1).

Mitotic index was determined as a percentage of the number of mitotic cells in relation to the number of counted cells.

The results of determination of the mitotic index of cell cultures exposed to the action of different propolis fractions are presented in Table No. 1.

Table No. 1

MITOTIC ACTIVITY OF CELLS EXPOSED TO THE ACTION OF DIFFERENT FRACTIONS OF PROPOLIS AND CONTROL CULTURES

Sign of fraction	0,5%		1,5%		2,5%		Controls (pure cultures)	
	number of cells	%	number of cells	%	number of cells	%	number of cells	%
B	800	9.4	350	6.7	700	5.1	600	6.2
D	400	6.3	400	6.1	400	5.9	600	5.9
E	600	11.2	600	13.1	600	8.4	600	6.8
Controls (ethanol)	300	6.4	400	4.5	lifeless culture		lifeless culture	
							Mean (control)	6.3

From our results the existence of differences in the mitotic activity between explored propolis fractions is evident. The fraction E shows twice as much intensive mitotic activity than fraction D, or controls. Ethanol in doses from 0.5% does not have essential influence on mitotic index, compared with the controls.

These propolis fractions did not cause clear chromosomal aberrations, except insignificant and usual wrinkle and lesions of chromatids.

It seems that our results are in agreement with data suggesting a regenerating influence of propolis on some tissues.

V. THE USE OF PROPOLIS IN MEDICINE

PROPOLIS

N. IOIRISH
USSR

From ancient times bee glue, called propolis, has been frequently used in medicine.

Empiric medicine uses it even today in the treatment of malignant tumours and wounds. During the Boer War it was often used in order to heal wounds and the results were excellent.

In 1901, N. ALEXANDROV published a small article "Propolis as Medicine", which dealt with the treatment of difficult wounds with propolis. This method had been known by him since 1893.

It is as follows : one piece of melted propolis is applied in the dressing. In a couple of days its root and crust are dropped. Nowadays, propolis is placed among the first grade remedies in empirical medicine.

In the World War II, at the proposal of L. HANDROSS, master in sciences, propolis was experimented with in two surgical hospitals, in Sverdlovsk. According to him, the treatment of wounds with propolis gave good results.

V. KIVALKINA, master of veterinary sciences, has recently showed that propolis has good bactericidal value as regards streptococci, staphyllococci, pyocianyc bacillus, colibacillus and other microbes. KIVALKINA's experiments were carried out to study the effect of propolis different species of bacteria (pathogenic, saprophytes, bacilli etc.).

Starting with melted propolis, 3—5 mm thick blades were prepared, on whose surface one placed drops from a culture of microbes studied previously for 24 hours.

From time to time one proceeded to culture media inseminated with peptone broth and peptone gel.

K. GAPTRAKHIMANOVA has successfully used propolis ointment when treating animals with necrosis. She concluded that the healing effect of this ointment (prepared with vaseline, sun-flower oil and henbane oil in the proportion of 1 : 1, 1.5 : 1) is superior to that of other medicines used.

N. TOPOROVA and I. TOPORINA used propolis for healing cattle necrosis. The experiments conducted on 9 species, where the disease was manifest by the mortification of some species due to the effect of *Bac. necrophorus*, led to a good therapeutic result.

After seven days of applying propolis ointment, one found that the necrosis disappeared and appearance of fresh granulations which showed quick healing even before the necrosis had gone. The ointment was made out of propolis and vaseline in equal parts. Propolis facilitates the normal nourishing of the tissues.

They also use propolis for wrapping around the corpses of their enemies (lizards, mice etc.) which enter the hive, thus removing any decay.

PROPOLIS, NATURAL SUBSTANCE — THE WAY TO HEALTH *)

K. LUND AAGAARD
DENMARK

Scientists appreciate that the species *Apis mellifica* is at least 42 million years old. It is an interesting fact that during this considerable period the bee, as opposed to some other animal species, which have experienced changes, even over shorter periods, has not altered, in one direction or another. This stability has had a great significance for the plant kingdom, which, without pollination whould have changed its appearance. The reason for this lack of change is a question for scientists.

The famous French scientist, professor Remy CHAUVIN, making some research on bacteria in 1965—1966 which do not infest insects, found that, in comparison to other insects, bees are free of all bacteria. On nutrient environments, where pathogenic agents should have multiplied there was no modification. After resuming the experiments twice, he came to the conclusion that bees occupy a special position among all other insects, being very seldom attacked by bacteria and viruses.

He turned then to research in order to discover the cause of this interesting phenomenon, especially with the aim of finding a new panacea which could assume the characteristics of penicillin, and of sulphamide drugs. At last he found that the bees secrete an antibiotic which make them resistant to any virus or bacteria. This was the moment which revealed a series of minute revelations of the life of the bee.

The research showed that *Apis mellifica* produces over 7 kinds of antibiotics : a substance which protects the body of the bee, the honey, the wax, the royal jelly, the pollen, the propolis and the venom.

The quality of being able to defend itself against any pathogenic agent is extremely important for the bees, when we think of the conglomeration of individuals within the bee colony (on less than 50000 cm^3 there live in each hive 50000—60000 bees). At such density, an infectious disease would have disasterous effects.

*) The title is that of a booklet edited in Danish, Swedish, German and English. We have selected the most interesting paragraphs (N.E.).

Propolis is the product with which bees cement their hive in order to protect the colony from draught, moisture and microbes.

The word propolis is of Greek origin, suggesting the idea of "defence system of a town".

The bees gather the raw material from poplar buds, and horse-chestnut when the weather is warm. The resin from the buds is mixed by the bees with secretions of certain glands. They obtain propolis in order to cover any vent in the hive walls. But they deposit it, in general, at the entrance : thus a propolis wall is formed, over which all bees pass when entering or getting out of the hive. So the propolis not only tightly cements the hive but also protects the bees colony against viruses and bacteria.

A revaluation of this quality of the bee was the pollen drug CERNITIN, which has a certain antibiotic effect. It was proved however that many unknown factors hindered a subsequent revaluation of the discovery.

All apicultural products upon which research was carried out in 1965—1966 were in fact also known and used as natural products in ancient times. For instance, one barns that propolis allowed trephination without the danger of infection. The Quoran also mentions propolis, presenting it as a different coloured substance.

In the Inca Empire, around 1600, before its destruction by the Spanish conquistadors, propolis was known, being used against swellings accompanied by temperature. But about 100 years ago, the interest in propolis disappeared, and it is not mentioned in lexycons and dictionaries.

In 1807 the substance is mentioned once more as a means of curing wounds, but in connection with honey. During the Boer war, 1899—1902, propolis was mixed with vaseline, the product obtained being called "Propolis-Vasogen". This gave good results in surgery. For centuries monks used propolis for healing.

The great development of medical science since the beginning of the XXth century has pushed propolis in to oblivion. In Denmark, the word "propolis" was wiped off from all dictionaries which appeared after 1900.

Rediscovery of Propolis

I became a beekeeper against my will, when I received a hive of bees as present from a friend when I moved to another town, a hive with 60,000 bees. With the help of some experienced beekeepers, I became an expert in this field. In two years my apiary increased to 6 hives.

By watching the bees activity all the time I could distinguish between the production of wax and that of propolis, repeating the experiment of another beekeeper who also noticed that the bees were gathering a claysing substance from certain trees. That beekeeper tried to dissolve it, afterwards smearing the inner cover with the solution. I also tried to dissolve the propolis. With much difficulty and using a sufficient quantity of water, I obtained a yellowish liquid. I smeared

some frames with it and placed them in colonies : the bees built comb better in these frames than in those not smeared.

The propolis produced by the bees from my apiary had a very particular, sweet perfume.

In 1966, the press items about the scientific discoveries of Prof. R. CHAUVIN in connection with those 7 antibiotic substances of the bee appeared, and thus I got some ideas about the correlation between bees behaviour and propolis.

On June 3rd, 1967 I experienced an incident which led subsequently to unexpected consequences of great importance. Although I did not feel very well because of some throat trouble which I had for a couple of days, I was obliged to go on a trip. At supper, in the evening of the same day the swelling in the throat became so annoying that I could not eat anything and I also found that I had a temperature of over 40°C. I decided to try some propolis which I have been collecting for many years.

I crushed the dried propolis in a mortar, dissolved the powder into a cup with warm water. I filtered it through a coffee filter and obtained a yellow infusion like tea. I gargled with it two or three times and drank the rest and then I went to bed. In the morning I found myself in perfect good health. I had only a slight red spot in my throat which disappeared during the day. I drew the conclusion that propolis contains a very strong antibiotic if it could cure such a swelling in 5—6 hours.

It was quite natural that I should tell what happened to my colleagues in the office. In a short time I had the opportunity to apply and verify this antibiotic on a colleague of mine who also had a throat swelling.

The news that I had a miraculous substance spread and some of my acquaintances tried this remedy for the throat for themselves and every time it was successful. But I became more careful, when I learned that propolis had an allergy phenomena. I was afraid that somebody would have trouble with respect to allergy when using propolis.

In the summer of 1967 I had a painful catarrh of my eye. The drops for the eye prescribed by the doctor did not cure me. When I saw that the sickness still persisted, I tried to treat it with propolis. I crushed a little dry propolis into a mortar, then dissolved it in warm water. I dripped this solution in my eye with a dropper. The eye smarted a little but I stood it. Next morning the catarrh had disappeared.

The examples mentioned were followed by others later on.

The Vast Experimentation

Between the years 1967—1973 I conducted 5 series of experiments which involved about 16,000 persons all over Scandinavia. There was a great deal of publicity about propolis at that time.

It also happened that I was very interested in propolis, as a hobby, long before I finished my experiments, I was visited by representatives of the press who wanted to know more about this research work which was so out of common. Many articles were published and read with

great interest by many people, and among them those declared by physicians as having incurable diseases.

We must mention that a normal colony of about 60000 bees produces annually only about 35 grammes of propolis. It is clear enough that a vast organization is needed for harvesting sufficient propolis so that thousands of people could try this natural curative substance.

Looking into the results of the scientific researches conducted on bees, I found that many researchers had discovered the antibiotic effect of the substance.

Based on close cooperation among beekeepers these experiments involved in 1974 more than 50,000 persons.

After discussing the matter with many experts from my country, I get the impression that the research on this product is very difficult.

It also implies that big investments of money for research will be required over a long period. During my long practice as a beekeeper I had the opportunity to examine certain affections and I often experimented propolis in its natural form and I obtained relevant results for the cases treated. In any case, propolis is not harmful, this being found out in one of our biggest hospitals.

The true experiments began in the autumn 1971, starting from the conclusions of the 3 series of experiments conducted between 1967—1970. Then, we found that the propolis may be divided into 5 categories : a) big ("grande") ; b) pelleted ; c) powder ; d) solid ; e) propolis aqua.

This division proved to be very important for establishing the active part of the substance.

The fourth series which included about 1,700 persons gave us amazing proof. Since I know at present the results of 16,000 cases all over Scandinavia I assume that propolis is indeed a substance with very big effect in curing or improving the condition of those suffering with nose, throat and ear trouble as well as of some diseases of other secondary activities. It will also heal some other diseases caused by viruses and bacteria in the region of the head. Meanwhile, propolis added to food gives good results in urinary tract infections.

Some letters containing information about the results obtained in the treatment with propolis, were considered. In 97% of the cases propolis gave positive results whereas 3% were negative. In 3 of the cases allergies occurred so that the treatment was stopped. Many experiments show that propolis has influence on the balance of the hormones. The health of patients having pernicious anaemia was improved by stimulating the production of red cells.

In cooperation with many specialists, among them Dr. Edgar WILSON from Massachussetts, U.S.A., they confirmed my observations in connection with a special ointment which contains 10—12% propolis. Both in Romania, Denmark, and U.S.A., there are proofs that wounds of the tibia, infections and swellings of skin were amazingly influenced by the treatment with propolis.

A report about my experiments and results was published by dipl. eng. Hans Erik CHRISTENSEN and Jens H. NIELSEN from the

Research Laboratory of the "Niels Steensens Hospital" and "Nordisk Insulin Laboratorium".

Eng. Hans Erik CHRISTENSEN made chemical and microbiological examinations in 1972 on propolis, at the Faculty of Technique in Denmark.

He demonstrated that this substance has special effects.

In their work, NIELSEN and CHRISTENSEN mention that according to the experiments performed by LUND AAGAARD cures were obtained, in many cases, of the following diseases : throat swellings, catarrh of the eye wounds, chronic gastric diseases as well as those caused by infections, swelling of the kidneys, of the bladder and of the legs.

What matters is Success

This chapter is an objective analysis of 220 letters chosen at random and they could represent the average from the whole collection of letters which report about the use of propolis. The authors of the letters are pe ple of widely different professions : a stoker, a medical nurse, two salesmen, a topometrist, a high-school teacher, a sketcher etc. Of those who wrote these were twice as many women as men.

214 letters (97%) reported a positive result.

In 3 of the cases allergy symptoms were found.

In another three cases the substance had no recorded effect. The field of influence of propolis is extremely broad : swelling of the large intestine, catarrh of the eyes, infection of the urinary tract, swelling of the throat, gout, open wounds, sinuses swellings, cold, influenza, bronchitis, gastritis, cancer. One also mentions cases of : diseases of the ears, parodontosis, caephalea, intestinal infections, micoses, ulcer, eczema eruptions, pneumonia, arthritis, lung disease, stomach virus, headaches, Parkinson's disease, bile infections, sclerosis, circulation deficiencies, warts, conjunctivitis, hoarseness, chilblains, etc.

„He was Cured"

On August 15, 1973 the headline *"He Was Cured"*, appeared in the regional newspaper Frederiksborg Amts Avis, Hillerod, dealing with the wonderful therapeutical characteristics of propolis.

The patient was 42 years old, he was born in Nordseeland and lives now in Bremen.

In 1969 he realized that he had cancer and that he would have to have an operation. He says : "After my wound was healed they made me have 36 Cobalt ray sittings. Then I was sent home and could eat only liquids. My weight was only 54 kg and I had two wounds in my mouth, which the physicians tried to heal with medicines. Because the situation did not improve after 8 months, the physicians decided that they would operate on me in a month's time".

The patient obtained propolis from a relative 6 days before operation. On being examined before operation the doctors noticed that the

ulcerations previously operated on had completely dried and could be easily removed with tweezers. The operation was now considered unnecessary.

"Thus discovering the effect of propolis I went on taking it. I was able to resume my work and regain the 20 kg which I had lost. After six months I stopped taking propolis. I had a break of 7—8 months".

After finding a malignant swelling of the pancreas, treated in hospital by normal methods and radiotherapy, the patient resumed the treatment with propolis. When the physicians asked him whether he had taken the medicines prescribed by them he answered that he had only taken propolis. There was a surprise when learning that propolis was the only medicine he took and that today he can eat and drink anything even a glass of beer or plum brandy. For prophylaxis and maintenance he continued to permanently take half spoonful of propolis every day, together with food.

Directions for the Use of Propolis

Propolis may have different colours and different smells depending on its origin, that is relative to the species of trees it comes from, as well as to its degree of maturity.

One recommends the use of a good quality and verified mixture. One has to mix up the grades "Grande" and "Solid Propolis". It has been proved that coming into contact with the enzymes from a person's spittle, propolis releases a strong antibiotic in a few minutes. The working quality of the substance is activated particularly by the warmth and moisture from the mouth. In order that a corrosive effect should not appear on the mucuous membrane of the mouth, the patient should have a rest of one or two hours after starting to chew the propolis. Then he should resume chewing until the substance crumbles and it can be swallowed.

One should eat 1—3 grammes daily. The spittle mixed with propolis can be used in many circumstances : treating wounds, burns, inflammations of the skin. The result is often surprising.

"Solid propolis" is used especially in the case of parodontosis ; during chewing, the substance becomes very adhesive and it deposits on the teeth ; it "works" when one is asleep.

The grades "Pelleted" and "Propolis powder" are taken depending on circumstance in doses of one spoonful (1—3 times per day).

The patient must take the corresponding quantity in the mouth and he should rinse the mouth with a cold liquid, after meals.

"Propolis-powder" is, as a matter of fact, the raw material for different ointments with various propolis concentrations. In the near future propolis will be sold in pills with vitamins and minerals having an addition of propolis named "Propolin".

Besides the minerals and vitamins they contain, the pills also have a refreshing effect both for regulating the hormones and as an antibiotic substance which is in itself a stimulator of the natural resis-

tance of the body. This is why the pills may be used by everybody, sick or healthy, as means of protection against microorganisms.

Very soon a special suppository for patients with intestinal diseases may be available. The numerous results of my experiments in this field, clearly indicate that a concentration of propolis in the rectum cures or improves the health of the patient.

The ointment has good effect in the case of eczema, of scars, etc. The ointment is prepared in two ways, with a different propolis content, one containing fats and the other containing water.

In the case of infectious diseases propolis is an exceptionally good addition to daily food. In these cases a medical prescription should be strictly followed.

In order to avoid allergy when taking propolis for the first time, one recommends a small portion for the first day before going to bed. If next morning one does not notice an unpleasant symptom, the treatment with propolis may begin.

— Infections in the region of the head : they are helped and very often cured with propolis as well as infections in the mouth, from the throat to nasal sinuses and other cavities.

The substance also relieves swellings of the ear. It was also proved that meningitis is cured in a short time. Usually the different infections of this kind may be cured in 4—8 months by using 20 grammes of the "Propolis grande" mixture. In the case of otitis, the quantity has to be bigger. But the more frequent forms of throat infections can be cleared up with a single dose of "Propolis grande" (for chewing) : if these troubles happen early in the morning they are cured in a couple of hours. When there is an acute case, the patient has to chew propolis all day long. Next day, the quantity of propolis may be dicreased. It is recommended that a dose should not exceed 5 grammes.

— Infection of the urinary tract : almost all infections of the kidneys, urinary tracts, bladder, prostate and the genitals are positively helped by propolis as an addition to the meals. "Propolis grande" and in more severe cases also "pellet" or propolis powder are recommended.

The effect is often very quick so that the troubles disappear in a couple of hours, or a couple of days.

For gastro-intestinal infections or affections the best grade of propolis is "pelleted propolis" added to the food although it may be replaced by "propolis grande" or "propolis powder". The quantity is from 2 to 8 dose, each containing 20 grammes. The substance has to be taken after the meals.

For the simple cases, a portion of 10 grammes consumed over 3 days may be sufficient for a cure. For chronic infections, as well as the microbial ones, in the case of internal organs need a bigger consumption of propolis between 40 and 100 grammes.

The propolis is also efficient in a series of affections connected with the lungs, blood circulation, skin, as well as other affections partially caused by bacteria, viruses or different fungi. For all grades of propolis, the beginning of the treatment must be performed with cau-

tion, gradually increasing the consumption over the following 3 or 4 days, until the maximum dose, recommended for the respective disease. After that, the consumption may be regulated by each patient.

Although one has to take into account the fact that propolis is not harmful it is however strong and may cause certain troubles, for instance, the irritation of the mouth cavity or scores.

It is therefore better to accustom oneself for 3 or 4 days. It is also important that the consumption should be gradually decreased after the cure.

Consumption should cease after 8—14 days. Propolis cures almost all diseases, because it is a special natural substance with strong effect, although there is no explanation for it on a scientific basis. Because it is not dangerous, it can be tried by anyone.

Preparation of Propolis

Propolis as a raw material is taken from beekeepers on a contract basis, for use in the laboratory. Here the most scrupulous hygiene is observed in cleaning, grading, mixing and preparing it.

Propolis may also be used in its natural condition or after a mere cleaning.

But I like to see that the propolis arrives to the consumer in an attractive form. It has had a guaranteed purity and it should not contain any of the hive impurities.

The purchaser must always find the same quality.

The whole research programme, with thousands of cases, had a single purpose, namely, to obtain a substance with the greatest efficiency against the greatest number of diseases mentioned.

It is very important for one to know the origin of the propolis, namely, the species of trees from which it comes.

The numerous healings are relevant by themselves and the number of people who use propolis is ever increasing.

It is my duty to draw one's attention on those bad replicas or falsifications which are sometimes put on the market by unscrupulous people.

During our trials we did not think of our enterprise as one that would require us to have to buy from other sources ; but the propolis gathered sold quicker that it could be collected. By being a cooperative, we succeeded in founding a solid organization which deals with the harvesting of propolis and of obtaining the best quality.

Although today, propolis (marked with a certain grade) may be bought in certain chemist's, drug stores and "Reform" shops (where they sell only natural products) as well as in Nordisk Propolis AG, this does not mean that the research work has come to an end.

My enterprise keeps up research into propolis as addition to food with the hope that this substance will soon be recognized as a medicine. Finally, it is very important to follow correctly directions for the use of propolis.

PROPOLIS — AN EFFICIENT TREATMENT

J. K. LEIPUS
USSR

At the XXIII[rd] International Apicultural Congress in Moscow, propolis was very much discussed.

The chemical analysis showed that the comb cells where brood develops are lined with propolis which protects brood from different microbes. Thus, the main role of propolis is the protection of bees against diseases.

The antimicrobial effects of propolis were found on different minor elements.

It was also observed that propolis destroys some minor elements, sometimes in 15—20 minutes and sometimes in 5 hours. This effect depends on the sensitiveness of the minor elements and on the propolis concentration.

Bearing in mind the fact that propolis also diminishes pains and swellings, it is used in our country as a medicine.

Many patients were cured by the use of propolis.

Propolis can be efficiently applied to catarrhs of the respiratory tract, in influenza, sinusitis, laringitis, bronchitis, asthma, chronic pneumonia, tuberculosis.

These are the diseases of almost half of the population of the world.

Sick children are very responsive to the treatment.

At present we use propolis in curing different surgical wounds in abdomen and gynaecology. It is also good for skin diseases (eczemas, keloyde, scars, psoriasis).

In stomatology apical granulomas may be healed. It may also be used in nose, throat, and ear diseases and especially in the treatment of the medium ear, otitis.

Propolis also heals the wounds of internal organs, thus destroying harmful toxins.

In all these diseases I used a propolis alcohol solution (20—30%) diluted in hot water and given 2—3 times a day, one hour before meals.

For the upper respiratory tract children are given propolis twice a day before lunch and supper. In the morning, children are prescribed inhalations with propolis for 3—5 minutes.

In the treatment of these diseases we used an alcohol swab with 30—40% propolis or propolis ointment 10, 20, 30%.

It is recommended that after healing and a short interruption, the treatment should be repeated.

TREATMENT OF COMMON CHRONIC RECURRING APHTHAE WITH PROPOLIS

M. GAFAR
Alexandra MÎNDRU SĂCĂLUȘ
ROMANIA

The common chronic recurring aphthae are included nosologically in the large group of vesiculary stomatites, an affection specific to man with a pluralistic etiology (during the last decades numerous scientists inclined to 2 different hypotheses : selfimmune and virus).

As a rule common chronic aphthae become localized on the mucous membrane of buccal cavity, namely :

— the vestibulo-jugal region
— on the edges and frenulum of tongue
— on the labial mucous membrane, more rarely at the level of gums, palate, mucous membrane of pharynx, larynx, oesophagus, gastric mucous and genital membranes.

The main features of this affection are : regular, sometimes seasonal, appearance in both sexes but particularly in children.

Among the causal factors those existing in toxic and chemical media which bring about psychical stresses are prevalent.

The affection breaks out suddenly by a slight feeling of smarting without fever and local or regional adenopathy, which differentiates it from other vesiculous affections localized on the mucous membrane of buccal cavity.

Both *clinically* and *histopathologically* common chronic recurring aphthae show identical development stages irrespective of their seats, namely : macula, vesicula, ulceration and formation of epithelium. These stages alternate rapidly, but the stage of ulceration is easy to detect by the physician and the patient himself, because it causes pains to him particularly when fed on seasoned meals.

From a histopathological point of view aphtha is considered as a "little infarct", its pathobiotic aspects evolving in successive stages irrespective of the origin of pathogenic agent ; it becomes localized at the level of vascular structures of corium bringing about endotelitis lesions which progress assuming angio-teleangiectasic aspects.

Local treatment of common chronic recurring aphthae

Given their non-specific etiology the treatments administered locally and *per os* are also different and aim at annihilating pains and favouring the formation of epithelium.

There are numerous caustic or slightly antiseptic substances which play a certain part in the evolution of these affections among which we mention : salicylic acid (20%), trichloracetic acid (3%) in glycerinated medium, zinc chloride solution (20%) and (30%), silver nitrate solution (5 to 10%), the nitrate pencil being well pointed. All these are applied strictly on lesions, the epithelium round them being protected.

Among coloring matters are : methyl-thionine-chloride (1%) or gentian violet solution (1%).

Similarly some authors recommend to cover aphthae with an adhesive paste. This contains pectine and cellulose which make it adherent to the mobile mucous membrane thus separating aphthae from the local medium and alleviating pains. The American authors added to it triamcinolon and volon which have a therapeutic antiallergic, antiinflammatory action, but they did not obtain satisfactory results.

In aphthae with *multiple lesions* are also indicated gargles 3 to 5 times daily with 1/10000 potassium permanganate solution or 1/2000 rivanol solution in infusion of *Matricaria chamomilla* by associating 1/500 xyline and 10% glycerin ; buccal baths with alcaline solution (one teaspoonful of sodium bicarbonate in one glass of lukewarm water ; one can apply local sprays with hydrocortisone (0.25%) (10 vials of 25 g each = 250 mg) to 100 ml of infusion of *Matricaria chamomilla ;* tablets of 25 mg hydrocortisone each held in the buccal cavity round the lesions (MORRIS, OWEN) repeated 3 to 4 times per day resulted in healing small and separate lesions in 24 to 48 hours. This brings about a rapid alleviation of pains and avoids the risk of corticotherapy *per os.* Besides this medication one can use sometimes with good results different medicinal substances administered by sublingual injections, such as vitamin C ; pyridocine, cyanocobalamine, vitamin C + vitamin A ; hydrocortisone, procaine or xyline 2% are indicated in stubborn cases to the previous treatments ; similarly one can use penicillin C associated with xyline 2% and other substances applied as sublesion infiltrations and others.

General treatment

In recurring lingering cases it is advisable that the above mentioned treatment should be associated with a general treatment with vitamins belonging to group B (pyridoxine, cyanocobalamine) ; follic acid, vitamin C and PP ; they can be administered *per os* as well as intramuscularly, being considered as component parts of the basic tissue systems involved in the acidifying of nutritive substances necessary to a normal function of tissues ; we also mention the therapy with procaine (2%) administered by i.m. injections, which acts on the trophical function of tissues and in part on the protective humoural non-specific factors ; the therapy with immunoglobulin which acts on the protective capacity of the body ; the steroid therapy (ACTH in glucocorticoids) which plays an important part in the general metabolism, it being antiphlogistic, antiproliferous, antianaphylactic and anti hyperglycohemia ; finally, antiallergic medications such as : Romergan, Viadril etc.

Nevertheless it was found that the application of a single procedure of the general treatment did not offer a favourable solution of cases because recurrences were frequent. That is why the combined local and general therapy was preferred according to tests as the case might be.

Results of investigations

In establishing the therapeutical treatment we took into account some aspects of the application of preparation ; thus we established a symptomatic (anaesthetic) treatment ; one etiologic (in cases where a microbial agent was discovered) ; a treatment stimulating the cicatrizing process and the formation of epithelium ; a general treatment for improving the reactivity of the body by specific and non-specific means.

Taking into account these landmarks we integrated the personal casuistic including 110 patients 30 of them being subject to a local and general treatment very well described by the specialized literature consisting of 3 usual caustic substances : trichloroacetic acid (3%), zinc chloride (30%), silver nitrate pencil (well pointed), which were applied strictly on lesions.

We acted first by a local treatment and to this end we established groups of 20 patients each treated with the above mentioned substances aiming at influencing in the first place the symptomatic effects.

Our findings are as follows :

— by using a 30% zinc chloride the painful sensations both tactile and those induced by contact with seasoned meals disappeared in 48 hours and the patient was cured in 7 days ;

— the application of trichloroacetic acid (3%) resulted in the disappearance of painful sensations in 24 hours and the complete cure of lesions took place in 7 days ;

— by applying the silver nitrate pencil we found that painful sensations disappeared in 12 hours and the cure of ulcerations occurred in 5 days.

At the same time we established a general treatment particularly for those with monthly recurring pushes consisting of the administration for 20 days of Complex B vitamins, 3 tablets daily, vitamin C_{200}, the same dose. After this interval we found an improvement in the evolution of affection, the frequency of recurrences diminished, these appearing at 2 months intervals.

At the same time the patients were recommended to observe a non-seasoned hygieno-dietetic regimen, the dairy products being excluded during the treatment.

Considering the results were not conclusive we went on studying a group of 80 patients by applying locally a preparation with propolis, an alcoholic extract. As is commonly known the specialized literature shows that propolis is widely used to treat different affections such as in O.R.L. rhinites, sinusites ; in dermatology — eczemas caused by staphylococci and streptococci, in the affections of the breathing apparatus (pulmonary tuberculosis) ; in burns of degree I and II ; in chilblains ; in oncology (certain tumorous forms) ; as non-specific ways of stimulating immunological processes ; it has also a wide application in veterinary science. In stomatology it is used as an anaesthetic in buccal-maxillary face lifting when it increases the effect of novocaine ; in parodontopathies and also in common chronic recurring aphthae.

Propolis occurs in a native state as a flavoured sticky substance (due to resins). It is dark yellow-reddish or dark green having a bitterish taste. Its melting point is over 80°C, its density — 1.27. It is insoluble in water but soluble in alcohol, ether, chloroform. It is found in the buds of poplar, chest-nut tree, cherry, peach tree, alder and common ash.

As to its chemical composition it contains flavoured substances (balsams), vegetal resins (benzoic acid — 20%, cinnamonic acid); flavone derivatives including chrisine, galangine (with antibiotic properties), quercitine, tecpinine, aromatic aldehydes such as isovanillin (an isomer of vanilla), acid groups (cinnaric, caffeic), alcohol (cinnaric alcohol), sugar substances (melesitose, glucose, fructose), chemical elements (iron, copper, magnesium, manganese, cobalt, sodium, potassium, calcium, aluminium, silicon), and bees wax in proportion of 30%.

Therapeutic properties

It has a bactericide and bacteriostatic action on gram positive and negative bacteria; it has antiinfectious properties (thanks to its antibiotic content); it is antifungal, haemostatic, analgesic, antipruriginous, cicatrizing, viruscide etc.

Nowadays there are alcoholic extracts, tinctures, fluid and soft extracts, solutions and ointments of propolis.

The alcoholic extract was prepared as follows: 40 to 100 g of fresh crude propolis cut in fine chips with a bistoury were put in a dark coloured bottle with ground stopper, after which alcohol 96% was poured in. It was kept for 10 days at room temperature, being stirred 3—4 times daily. At the end of this period the solution got dark orange to brown. It was filtered through a paper filter and cotton-wool, which determined the reduction of its initial concentration of 40%, the pH being in the neighbourhood of 6.

The solution can be kept for a long time in a place safe from light in dark coloured bottles with ground stopper. It is applied daily on lesions with the help of a small brush or sterile cotton-wool tampons slightly imbued with it, after previous tamponing of ulcerated zones by sterile compresses well insulated and pressed for 3 to 6 minutes in order to obtain a protective film on the surface of lesion. The patients are recommended not to eat for 1 to 2 hours.

In more serious cases with short recurrences we associated a general treatment to obtain both immediate and late effects on recurrences.

The results obtained with the 80 patients under observation were as follows: in 85% of cases the pain disappeared in 2 to 4 hours; in 10% of cases the pains yielded after 24 hours and lesions were cured in 3 days, in 5% of cases the results were poor, the lesions being cured in 6 days.

As to the action of preparations on the recurrences we found that in 85% of cases watched for 1 year the interval between pushes was extended from 1 to 3 months to 4 to 12 months. Given the different causes of affections in these cases, we administered also a general treat-

ment with vitamins B (3 tablets/day), vitamin C_{200} in the same dose, for 15 days monthly, with a break of 1 month.

In 12% of treated patients, the interval between pushes increased but in 6% of cases, in spite of this complex treatment, the frequency of recurrences remained unchanged. Only the acute push was favourably influenced.

Conclusions

We think that propolis can be included in the local treatments depending upon the individual reactivity of each patient and certain disfunctional states.

We agree with the authors quoted in literature that the favourable action of propolis can be attributed to several mechanisms of its components such as: formation of a protective film on the surface of ulcerated lesions, suppression of the irritant action of external excitants as well as the strong anaesthetic action which annihilates pains and vascular spasms favouring the cure, or perhaps it contains some antivirus factors, this hypothesis being admitted nowadays in the etiology of chronic recurring aphthae.

Thus the treatment with propolis shows its effectiveness by alleviating pains and also as a factor which stimulates the local tissues. Its components are harmless in comparison with the other caustics when they are not limited to the ulcerous area.

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OBSERVATIONS ON THE ANTIBIOTIC EFFECTS OF PROPOLIS, POLLEN AND HONEY

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The bactericide effect of bee products has long been known. Recent investigations showed the inhibitory activity of extracts of such products prepared by various methods as well as their antimicrobial activity in the treatment of some surgical, dermatological and ophthalmological affections.

This paper gives the results of our observations on some microbial strains of animal origin made by an original method.

Material and method

In our experiments we used propolis harvested in 1975 and pollen and honey from the 1976 harvesting coming from the forestry zone Cozmești, Jassy district. Pollen and honey were used as such and propolis under the form of extract (hot and ethylalcohol 96° extraction).

a — Hot extraction : 6 g of propolis + 20 ml physiologic serum heated for 1 hour at 80°C ;

b — Ethylalcohol 96° extraction : 5 g of propolis + 20 ml alcohol kept at room temperature for 24 hours.

Working method

For all tested bee products we used ordinary agar-agar on Petri dishes 10 cm in diameter. In the agar-agar 6 mm thick there were made 4 cups in which a thin layer of gelose was put in order to prevent diffusion of products under the agar-agar layer.

Testing propolis : 3 drops of propolis extract were put in each cup and compared with penicillin 0.4 IU/cup.

Testing pollen : 10 granules of pollen were put in each cup.

Testing honey : 3 drops were dripped in each cup.

Readings were performed after 24 hours by applying the method used in radial diffusimetry of antibiogram.

Table 1 gives the behaviour of microbial strains of animal origin towards propolis.

Table 1

BEHAVIOUR OF SOME MICROBIAL STRAINS OF ANIMAL ORIGIN TOWARDS PROPOLIS

Series No.	Strain	Hot extraction method	Ethylalcohol 96° extraction method	Penicillin 0.4 IU/cup	Results
1	*Staphylococcus aureus* Ox.	1.2 cm	1.7 cm	1.5 cm	sensitive
2	*B. coli* (O_5)	—	—	—	resistant
3	*Salmonella* B	1.3 cm	1 cm	—	sensitive
4	*Salmonella* D	0.8 cm	1.1 cm	—	sensitive
5	*Pasteurella avium*	1 cm	1.2 cm	—	sensitive
6	*Proteus* sp.	1 cm	1.2 cm	—	sensitive
7	*Listeria monocytogenes*	1.2 cm	1.1 cm	—	sensitive
8	*B. anthracis*	1.1 cm	1.3 cm	1.1 cm	sensitive
9	*B. cereus*	1 cm	1.1 cm	—	sensitive
10	*Pl. tetani*	To 1/14 water extract dilution in Veillon gelose.		positive	resistant
11	*Cl. perfringens B type*	Does not develop in 1/14 water extract dilution in Veillon gelose.		positive	sensitive

As it can be seen in this table all strains save for *E. coli* and *Pl. tetani* are sensitive to water and alcoholic extract of propolis.

As a rule alcoholic extract is more active than water extract, which can be explained by an increase in the antibiotic effect of propolis by alcohol. In addition we notice that in the inhibitory zone of penicillin there appeared resistant colonies of *Staphylococcus* and *Bacillus anthracis* whereas in the zone of propolis there is no trace of resistant mutants.

In table 2 we give the behaviour of some microbial strains of animal origin towards pollen.

Table 2

BEHAVIOUR OF SOME MICROBIAL STRAINS OF ANIMAL ORIGIN TOWARDS POLLEN

Series No.	Strain	Inhibitory zone with pollen	Results
1	*Staphylococcus aureus* Ox.	0.6 cm	moderately sensitive
2	*Bacillus anthracis*	0.6 cm	moderately sensitive
3	*Bacillus coli* O_5	1.5 cm	sensitive
4	*Salmonella* D	1.2 cm	sensitive
5	*Salmonella* B	1.3 cm	sensitive
6	*B. cereus*	—	resistant
7	*Listeria monocytogenes*	—	resistant

One can see that all tested strains save for *B. cereus* and *Listeria monocytogenes* are sensitive and moderately sensitive.

Table 3 presents the behaviour of some microbial strains of animal origin towards honey.

Table 3

BEHAVIOUR OF SOME MICROBIAL STRAINS OF ANIMAL ORIGIN TOWARDS HONEY

Series No.	Strain	Inhibitory zone with honey	Results
1	*Staphylococcus aureus* Ox.	1 cm	sensitive
2	*B. anthracis*	0.9 cm	sensitive
3	*Salmonella* D	2 cm	sensitive
4	*Salmonella* B	3 cm	sensitive
5	*B. coli*	2 cm	sensitive
6	*B. cereus*	—	resistant
7	*Listeria monocytogenes*	—	resistant

Besides one can notice the appearance of 2 inhibitory zones ; one is clear but the other shows resistant mutants.

We are planning to test in the future more bacterial species and make observations on the antimicrobial activity of bee products in *in vivo* experiments with laboratory animals.

If the results are promising we shall try to put them into practice particularly to treat surgical affections.

Conclusions

1. Extracts of propolis were made by hot extraction with physiologic serum and ethyl alcohol 96° and tested for their effects on some microbial strains of animal origin. Honey and pollen were tested as such.

2. Propolis extracts were tested on aerobic and anaerobic germs ; honey and pollen on aerobic.

3. Propolis extracts were tested in comparison with penicillin 0.4 IU/cup.

4. By using the inhibitory zone round the cup according to criteria of radial diffusimetry as a reading method the following germs proved to be sensitive :

— to propolis : *Genera Staphylococcus, Salmonella, Proteus, Pasteurella, Listeria, Bacillus anthracis, cereus, perfringens ;*

— to pollen: *Genera Staphylococcus, Salmonella, Bacillus coli, Bacillus anthracis ;*

— to honey: *Genera Staphylococcus, Bacillus anthracis, Salmonella* and *Bacillus coli ;*

5. Propolis extract had no effects on *E. coli* and *Pl. tetani.*

Pollen and honey had no influence on *B. cereus* and *E. coli.*

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TREATMENT OF SOME INFECTIONS WITH PROPOLIS SOLUTION

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In this article we shall share our experience of using 5% propolis alcohol solution and ointment.

The 5% propolis alcohol solution is prepared as follows: 10 g of propolis is ground and the wax and other impurities removed from it. The prepared solution is put into a flask, is covered with ethylic alcohol (96%) — one part propolis and ten parts alcohol and is infused in the dark for 3 days at room temperature.

The solution is stirred every day, for 30 minutes. On the third day, the solution is reduced to 0°—5°C for 2 hours.

The upper level of the solution has a yellow-brown colour and a pleasant flavour.

The middle portion consists of undissolved particles of propolis and the bottom one, rough wax dark particles and other mechanic impurities.

The solution is filtered through filter paper. The rough part of the content from the filter might be washed with a small quantity of alcohol and it may be used for preparing the ointment (10%, 20%, 30%) in fat excipient. The ointment is prepared with anhydrous lanoline, oil or butter. The excipient is melted over a water bath and the propolis added (undissolved in alcohol).

From the upper filtered level of propolis solution, the amount of dry matter is determined with the help of a refractometer.

For a 5% propolis alcohol solution the refraction rate must be 1.375—1.377.

If this rate exceeds 1.377, the necessary alcohol is added and if this is smaller one adds a small amount of ground propolis. 100 ml of solution must contain 5 g of propolis dry residue.

In the case of infected wounds, a 5% propolis solution facilitates the wound healing process, a fact which intensifies the growth of peripheric epithelium.

The alcohol propolis solution is also applied in the treatment of respiratory tracts using aerosols. The inhalations are performed by means of an electric aerosol apparatus, AI-1 (dilution 1 : 2 or 1 : 3 in distilled water, boiled milk, peach or apricot oil). The duration is 1, 3, 5—10 minutes daily. The treatment consists of 25 dosages. After each dosage the patient has to take a rest for 25 minutes.

When this treatment is prescribed, one has to take into account the general counterindications of the physiotherapic treatment with aerosols. There are some cases, however, when this treatment cannot be tolerated.

There are also other cases when itching appears along with small swellings in the form of a rash. In these cases one recommends that the treatment be stopped. The astringent effect on the blood vessels is accompanied by anaesthesis of the mucous membranes.

On the surgical section of the hospital nr. 22 in Kiev, a 5% propolis solution was applied in aerosols and used in the treatment of some trophic ulcers which had a torpid evolution of the legs. The treatment was applied on 25 patients and all of them were cured.

A 70 years old patient fell ill in August 1970. After an excoriation on the right shank, a trophic ulcer was formed with the dimensions 3.5×5 cm. Medicines had no effect. The patient followed a treatment — 17 sittings of aerosols with propolis (1 : 2) after which the ulcer surface got epithelium. After each sitting, a dressing with ointment of propolis solution (1 : 2 or 1 : 3 in 1% novocaine or distilled water) was applied.

In the treatment of wounds the wound area was treated with oxigenated water then it was wadded with a sterile wad. Then a napkin or a wad with propolis was applied. The ointment stimulated the growth of granulations and of epithelium.

The 5% alcohol solution with propolis may be applied for the upper respiratory tract infections : rinitis, catarrhs of influenza origin, tracheitis, bronchitis, pneumonia as well as in the treatment of wounds.

Strips dipped into propolis solutions, wads with ointment and gargles were used for the above mentioned, too. Another method in use is the applying of the aerosole spray for 4 minutes (1 : 2 dilluted solution or 1 : 3).

EFFECTS OF PROPOLIS IN OTORHINOLARYNGOLOGY

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Between 1971—1972 we tried to use the anti-swelling, mucous, analgesic and epithelium growth effects of propolis in O.R.L. practice. The treatments applied had the following diagnoses and results :

Diagnosis	No. of patients	Result		
		good	improving	no improving
Diffuse external otitis	4	4	—	—
Chronic otitis with acute mesotympanitis	4	—	2	2
Traumatic perforation of the tympan membrane	2	2	—	—
Ulcer stomatitis	3	3	—	—
Foot and mouth disease stomatitis	6	4	1	1
Chronic laryngitis and chronic rhinopharyngitis	10	8	2	—
Osena	3	—	3	—
Stomatitis after amygdalitis operation	17	—	17	—
Bronchiectasis	2	—	2	—
Asthmatic bronchitis	4	1	3	—

In the first diagnosis we had 4 cases of external otitis with severe diffuse swellings which brought about painful itching in some of the cases. We applied propolis in alcohol solution (5—7%), 2—3 times a day with the help of a wad dipped in the solution and placed on the external hearing tract. Meanwhile we also gave antibiotics. After 5—9 days of treatment we recorded an improvement of the status.

The second diagnosis : in 4 chronic mesotympanal otitis we applied an alcohol propolis solution — 5% — 2 or 3 times a day (in dosage of 10 drops) in the external hearing tract and at the same time a wad with 7% alcohol solution was applied. The treatment with propolis was maintained for 8—10 days, also accompanied with antibiotics. In the two cases when treatment was applied without success *Klebsiella* and *Pseudomonas* were found. These were cured afterwards with antibiotics.

The third diagnosis : in two cases of triangle perforation of the tympan we daily applied a 15% propolis alcohol solution on the sides of the perforated area using a wad. This was performed twice a day.

The fourth diagnosis : in 4 severe ulcer stomatitis we applied a 3—5% propolis alcohol solution by wadding the mucous membrane before meals. At the same time, vitamins and antibiotics were given. After 8—12 days an improvement of the patients' condition was recorded who could eat easily as a consequence of the analgesic treatment.

The fifth diagnosis : we identically treated 6 patients with foot and mouth stomatitis. They were cured after 3—8 days of treatment depending on the case. Only one patient of this group had phenomena of dermic allergy and was cured by help of some oral antiallergic.

The sixth diagnosis : in 10 patients with chronic laryngitis and rhinopharyngitis, atrophic alterings of the mucous membranes were recorded.

These patients were treated with aerosole inhalations 3—5% propolis alcohol solution ; the inhalations were done 5—10 times daily or every two days. Even since the second or third inhalation, a gradual improvement of the symptoms was observed in all patients. A yielding of the mucous membrane swelling, an ease to expectoration and the disappearance of the mucosities were also observed. In this group of patients we did not resort to any treatment.

The seventh diagnosis : in 3 cases of atrophic pheotida rhinitis — osena we applied the following treatment :

a) 3—5% spray plus 5—15% ointment on gauze were put in the nose 1—2 times a day ;

b) aerosole inhalations once a day with a propolis alcohol solution (10—12 inhalations) ;

c) the treatment was combined with antibiotics, vitamins and iron in drugs.

The crusts and rough mucosities came out easily after propolis administration.

In the 17 patients with the eighth diagnosis they were applied a 5% hydro-alcohol solution after the amygdalitis operation. This was a spray applied on the palate of the mouth and on the operated surface

from the first to the third day after the operation, 2 to 3 times a day before meals for 2 to 5 days. A good cicatrization of the post operatory wounds was noted. Thus the propolis has a moderate analgesic effect.

In the ninth diagnosis, in 2 cases of bronchiectasy we applied propolis in aerosol inhalations 5 minutes per day. A gradual reduction of the expectorate and its consistency was observed.

In the tenth diagnosis, in the patients with asthma bronchitis, an improvement of their breathing and of the expectoration was recorded.

The propolis is thus, a complementary medicine also recommended in otorhinolaryngology.

THE TREATMENT OF HYPOHEARING WITH PROPOLIS

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Hearing inability and hypohearing are two terms which essentially suppose the same condition. Hearing inability is, as a rule, the result of an inborn insufficient development of the ear or the result of a hurt of the apparatus which records the sounds of the environment. It cannot be treated.

The hypohearing appears as a consequence of some pathological alteration of the auditive nerve under the influence of different external factors (noise, vibrations, traumas) and internal ones (chronic medium otitis, infectious diseases — measles, epidemic paroitidis, influenza, drug intoxications).

The hypohearing remains for the time being a largely spread affection. The treatment often leads to improvement but never to curing.

I used propolis extract mixed with oil, in the treatment of hypohearing.

In propolis, the vitamins B, PP, A, were found and its chemical composition is already known. When preserved, propolis does not lose its therapeutic features, keeping them from 1 to 5 years. However the most relevant characteristic therapeutic features are contained by fresh propolis.

It is known the fact that propolis is a remarkable therapeutic medicine with bactericide, antitoxic, antiswelling, analgesic and regenerative effects. Taking into account all these, I applied propolis in the treatment of hypohearing. I used a propolis alcoholic infusion 30—40% mixed with oil (olives or corn oil) in the 1 : 4 ratio.

By stirring the mixture one obtains a homogenous liquid light-brown and with a pleasant flavour. I made the treatment by introducing some gauze wads with propolis oil-alcoholic solution in the auditive duct.

In the case of children (over 5 years old) these are daily applied in the evening and kept 10—12 hours (10—41 repetitions) and in the case of adults, every 2 days kept 36—38 hours (10—12 repetitions).

I treated 382 sick people at home.

The etiologic factors of their diseases were the following : chronic medium otites (197 people), infectious diseases (26), drug intoxications (7), vibrations, noise (15), traumas (6), otosclerosis (15), senile hypohearing (25), other etiologies (90).

The patients were between 10 to 45 years old ; sometimes even over 45.

The patients were divided into 3 groups depending on the degree of their hypohearing. The first degree — perception of whispering at a distance of 0.5 m from ear : 156 patients ; third degree — whispering-zero and loud conversation close to the ear : 96 patients.

After the treatment, the hearing improved in 314 patients : 199 of them grasped the whispering at a distance of 2.5 to 5 m, and 115 — the whispering at a distance of 1 to 2.5 m ; in 21 of the patients the improvement was not significant and in 47 patients there was no improvement.

During the whole period of the treatment and after it was over, the general mood of the patients grew better ; in the case of senile hypohearing some of the patients recovered their memory.

The hearing became more sensitive after 4 to 6 repetitions and the definite healing took place after 8 to 12 repetitions. The following anamnesis is to illustrate the above-mentioned :

Patient B, 41 years old, a worker in a plant complained that she had roaring in the ears and she could not hear very well : she already had hypohearing for ten years. She worked all the time in noise and vibrations. In her childhood she had had suppurative secretions in her ears. Before treatment she grasped the whispering to right and to left if it were next to the ear.

The audiogramme indicated a diminishing of the hearing as concerns the direction of sounds from right to left and the reception of sounds with both ears, at high and low noise.

After the treatment the hearing reached almost the normal stage, that is she grasped whisperings 4 meters to the right and 5 meters to the left.

During the treatment we observed sensitiveness to propolis. The ear auricle and the auditive duct became flushed and there was the appearance of itching : these phenomena were present on the second or the third day after treatment.

The application of propolis alcohol-oil emulsion is not recommended in the case of polypus and of the granulations in the tympan cavity.

Despite the remarkable therapeutic features of propolis it should not be used without medical prescription.

TREATMENT WITH PROPOLIS OF ACUTE INFLAMMATIONS OF THE MIDDLE EAR

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The chronic suppurative affections of the middle ear are dangerous because their complications can bring about hearing inability. For 6 years we have been using propolis alcohol solution in the treatment of chronic suppurative affections and of acute inflammations of the middle ear.

Propolis broken into pieces is introduced into a glass of cold water ; beeswax and the mechanical impurities rise to the surface and propolis deposits at the bottom. The sediment is dried, and then covered by 70° alcohol (30 g propolis to 10 cu. cm alcohol). It is left to macerate for 24—48 hours, being stirred several times, after which it is filtered.

Between 1968 and 1973 we had 68 patients with chronic purulent secretion from the ear (the disease having set in 1 to 20 years before — 28 having epitympanitis, and 103 patients with otitis media. The patients were divided in three groups.

We point out that in the beginning we used propolis in the treatment of the patients in whom the treatment with sulphamides and antibiotics had no effect at all.

The first group included patients with epitympanitis. After thoroughly cleaning the ear from pus, small gauze tents soaked with propolis solution were introduced into the auditive canal, pressing them against the tympan. The treatment was made everyday, and lasted for 10—15 days on an average (according to the seriousness of the ailment). The affection was considered healed whether within one month — after the treatment was over — no secretion occured any more.

The treatment of the 27 patients with epitympanitis was more complicated. In spite of the thorough daily cleaning of the ear, the secretion would not cease, although the fetid odour maintained itself only

in 2 patients. A lavage of the epitympan of all patients of the second group was made ; also, a lavage was made of the middle ear of 3 patients with epitympanitis in whom the purulent secretion lasted for more than 15 days.

Lavages with propolis solution were made once to three times weekly according to the patient's tolerance and evolution of the affection. The longest duration of the treatment was 2 months.

After the treatment, in 13 patients the secretion stopped, in 8 patients only a drop of mucus would accumulate in the auditive canal, and in the others secretions have reduced significantly but did not stop.

Not all patients could tolerate lavage of the epitympan. An irritation appeared in the vestibule of a patient after the first lavage, and in another after the third lavage, so we had to discontinue them.

The patients in the third group — with otitis media — were treated according to the same method only when no haemorrhagic exudate occured in the middle ear, and when the tympan was not swollen.

When propolis solution was administered by means of tiny tents, the secretion process stopped for 2 to 3 days earlier than when alcohol solutions of boric acid, furacillin, or other alcohol solutions were used.

Before starting the treatment with propolis the patients were examined, polypus or granulations in the ear were carefully extirpated in order to restore the normal breathing process.

The results of the treatment with propolis showed that propolis has a positive effect. Only in 6% of patients (treated for 1 to 6 years) the chronic purulent otitis — developed after catching of a cold — was aggravated.

One month after the secretion had stopped, hearing recovered in 52 patients, 55% heard whispers from 5 m, 30% — from 2 to 3 m, and in 15% no improvement in hearing was recorded.

The alcohol propolis solution is recommended to be used in treatment of acute inflammations of the middle ear of out-patients under medical observation.

THE TREATMENT OF MESOTYMPANITIS WITH PROPOLIS

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Chronic mesotympanitis represents a severe social problem because on the one hand it might generate complications, and on the other, it might be dangerous for hearing.

This malady appears as the consequence of a suppurative swelling in the medium ear — having as background a severe infection, the

decrease of the organism resistance and even the appearance of a chronic catarrh of the upper respiratory tract.

The generating factor of this malady becomes resistant to antibiotics and sulphamides, a fact which complicates the curing of the disease.

Propolis alcohol extract has a bactericidal effect and it ceases the collecting of pus, increases the phagocytosis as well as the natural resistance of the organism. When heated it does not lose its features.

Pharmacological effects of propolis are a basis for the use of it in the treatment of suppurative chronic swellings of the medium ear — mesotympanitis.

Since 1966—1968 we have treated with propolis 40 patients with mesotympanitis in the Hospital No. 1 in Sofia.

The patients were employees of the industrial enterprises of Sofia and the treatment was administered at home.

In the suppurative secretions, colonies of strepto-staphylococci were present and were resistant to antibiotics.

Of the patients treated with propolis, 12 were women and 28 men. Until the beginning of the treatment 10 of the patients had already been suffering from the malady for 10 years, 16 of them for 3 to 5 years and the others for 3 years.

At the control of the tympan, 33 patients had a small size perforation, placed in the anterior, inferior and posterior cuadrant.

Through perforation a green-yellow matter seaped, having a characteristically unpleasant smell.

The hearing decreased in different degrees in all patients.

The treatment consisted of a propolis alcohol extract prepared as follows :

30 g of ground propolis, dissolved in 100 g of 95° alcohol for 24 hours. During the extraction, the mixture was stirred a couple of times. The extract obtained was filtered and thus we got a reddish transparent solution.

In the less severe cases or recent ones, after cleaning the pus from the ear, propolis was applied once a day in alcohol extract.

In severe cases, a wad with the above-mentioned extract was applied in the ear and kept there for 24 hours. The next day the procedure was repeated. The healing period was on average 10—20 days.

The result of the treatment was as follows :

In 32 patients (80%) a gradual decrease of the suppuration was recorded, also the disappearance of the unpleasant smell, the pus was no longer collecting and the hearing grew sharper.

In 8 patients (20%) the collection of the pus was reduced to a great extent but not totally. Usually these patients were not cautious and did not follow the entire treatment.

Seven of the patients were too far gone with the tympan perforation, they having only one part of the anteriour upper cuadrant and after suppuration had ceased remained with a poor hearing.

After the treatment was over, the patients were recommended not to catch cold, to keep away from getting water in their ears and to avoid virus influenza.

The treatment with propolis in chronic suppurative mesotympanites has a positive therapeutic effect and it is accessible every day.

A NEW DEODORANT

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By using propolis alcohol solutions for the treatment of 38 patients with chronic otitis, out of which there were 4 with granulomatous external otitis and 9 with pathologic condition of the cavity after a radical operation, I found that propolis has the characteristic of removing the unpleasant smell.

The unpleasant smell has an upsetting effect on the patient, thus producing depression; such patients avoid society, their sociability decreases, they would rather be alone. Sometimes they change their occupation and at other times they give up working. This is why the therapeutic and deodorant means are very important.

In order to confirm the above, we conducted experimental research.

The odorous content taken from the ear on a wad was put into a 20% propolis solution (in 40° alcohol). 40° alcohol was the control. The wads were numbered and in order to determine the extent of the smell, they were given to 5 persons with a good sense of smell. This kind of experiments was performed by using small pieces of meat (0.5 g) which had bad odour (20 samples).

In the first case and in the second, it was established that propolis can remove an unpleasant smell with alcohol.

This ability of propolis may be linked with its bactericide and bacteriostatic effect and also with its strong balsam smell.

Thus, our observations concerning the patients with diseases accompanied by an unpleasant smell and our experimental data confirmed the deodorant feature of the propolis alcohol solution.

PROPOLIS AND CHRONIC PHARYNGITIS

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The chronic inflammatory diseases of the pharynx mucous membrane are wide spread.

These patients often complain of dryness in their throat, smarting, the sensation of an alien body in their pharynx. They also expectorate spittle and bad smelling crusts, sometimes with blood. Some of them have headaches and even fever.

Propolis having antimicrobial, antiinflammatory and anaesthetic characteristics is very important for recovery in mucous membranes atrophic diseases. We used propolis also in chronic pharyngitis.

Ground propolis is put into a glass of cold water; while the wax and other elements rise to the surface of the water, propolis deposits itself on the bottom of the glass.

This sediment is dried by evaporation and 96° alcohol is poured over it (30 g propolis to 100 ml alcohol). The solution is kept thus for one week. It should be stirred now and then filtered. One part of propolis extract is mixed with two parts of glycerine (or peach oil).

In chronic pharyngitis the mucosities are first cleared and painted with the usual solution, once a day for 10—15 days.

For painting, 2—2.5 mg of solution is necessary.

We watched the effect of propolis on 238 patients out of which 187 suffered from underatrophic pharyngitis and 51 from trophic pharyngitis. Most of them were 33—67 years old, and they had been sick from one and a half to twelve years. 150 were women and 88 were men.

After treatment with propolis extract 74.6% of the patients were cured and in 14.7% there was a considerable improvement in health; 6.9% also improved more or less and on 3.8% the propolis extract had no effect. We observed the results of this treatment from 6 months to 3 years in 175 patients.

71.1% were completely recovered, 16.3% improved their condition, 8.2% felt better and in 4.5% it had no effect.

3 of the patients had allergy manifest by pains in the pharynx, a hard swallowing etc. Taking this aspect into account, it is necessary that before beginning the treatment the patient should undergo some tests concerning propolis.

Propolis appears to be essential in otorhinolaryngology and it has almost no counterindications.

NEW DRESSINGS FOR THE MUCOUS MEMBRANE OF MOUTH CAVITY ON PROPOLIS BASIS

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The swelling of the mucous membrane of the mouth cavity is a wide-spread disease, difficult to cure. The usual treatment is by means of different medicines but they are not always efficient.

In acute gingivitis-stomatitis, the best results are obtained with the so-called solid dressings in which case the antibiotics and the other medicines are maintained by means of dressings with zinc oxyde, zincoplast, stomacyd etc. The shortcoming of these applications lies in the fact that a great amount of solid substratum deposits on the mucous membrane and this is unaesthetic and unpleasant.

Another shortcoming is that these types of dressings are limited only to the gum mucous membrane and they cannot be applied on the tongue, or the palate and on the inside of lips and cheeks.

In the stomatology section of the Sliven polyclinics (Bulgaria) one obtained excellent results based on long experience by applying a new type of biological dressing on the mouth cavity mucous membrane containing an alcohol-etheric propolis solution. The advantage is that the alcohol and ether form a thin layer by evaporation which is aesthetic and confortable. This dressing persists for 24 hours, anaesthetizes the sick area and facilitates the effect of the active substance.

This new dressing was called STOMAPIN ("stoman" = mouth and "apis" = bee). Its main component is the propolis alcohol-ether solution, plus antibiotics, vitamins, colouring matter etc.

Way of Preparing

50 g of ground propolis is dissolved in 50 ml ethylic alcohol (70%) plus 20 ml ether. This mixture is preserved for a couple of days in a dark bottle corked tightly and stirred now and then. After dissolving it is filtered through a gauze and the liquid obtained is used as a basis for treatment. This liquid might be used as such, but if we wish to completely remove the solid substances, it has to stay for a short while and then the supernatant has to be poured into another flask and next added to the following composition :

Propolis filtrate	50 g
Rovamicyne or rondomicyne	
Honey with 2% royal jelly	5 g
Fish oil	2 g

The mixture obtained is homogenized and poured into dark bottles.

This liquid is very dense, of dark color, pleasant flavour and very volatile.

Before applying the dressing the mucous membrane should be dry. The yellow layer which will form on the mucous membrane persists for about 24 hours, the spittle dissolving it after this period.

However, it infiltrates into the mucous membrane.

Application

The infected area has to be cleaned with oxigenated water, then it should be dried with hot air and the prepared solution dripped on to it until a uniform layer is formed. Then the infected area dried once more with a slight air stream until the ether and alcohol evaporate.

Thus, the protection layer is formed. The treatment is repeated every 3 to 5 days until complete healing has been achieved.

Posology

1. In suppurative gingivitis with severe catarrh, in glositis, stomatitis.

Even after the first application pain decreases and the suppuration ceases.

The healing follows after 2—5 days of treatment.

2. In incipient parodontosis with haemorrhagia. After 5—6 days of treatment the bleeding ceases. The mucous membrane gets its normal colour.

3. In parodontal abscesses — gauze drainage and STOMAPIN.

4. In pains after extraction — the drainage of the socket with STOMAPIN dressing. The pains cease at once. The treatment should be repeated until healing occurs.

STOMAPIN is also efficient for mycoses and foot and mouth stomatitis.

STOMAPIN Varieties in Medical Theraputics

Stomapin + Nystatin

A great importance in the appearance of mouth cavity mycoses is presented by antibiotics especially those with a large spectrum. Some authors assert that the antibiotics stimulate the development of the fungus *Candida albicans* because they modify the bacterial flora of the mouth cavity. The more the fungus develops, the more severe the disease grows in the mouth cavity.

In cadidoses the antibiotics may not be administered, neither may STOMAPIN be applied, because it contains a strong antibiotic. The modern treatment in these cases is based on the alkalization of the mouth cavity by means of some colouring matters or of the mycostatic Nystatin. This antimycotic may be applied either internally or externally by smearing the mucous membrane with glycerine solution.

However the glycerine is easily removed by the spittle and this is why it should be often applied.

We obtained very good results by using STOMAPIN in the case when the antibiotic from it is Nystatin. The latter does not dissolve in the propolis alcohol-ether solution and that is why it should be stirred several times. Besides the therapeutic and analgesic qualities of propolis, Nystatin has a big effect and also a "retard" (delay) effect due to the dressing, but this treatment is a long-term one.

Coloured Stomapin

Another variety of STOMAPIN contains instead of an antibiotic a colouring matter with healing effect, as methylene blue, piocianyn (1.2%), Mirré ointment, especially indicated in foot and mouth stomatites

It is also indicated for children when antibiotics are forbidden.

Cases from Clinical Practice

R. P. D. : a 20 months old child, required treatment with a great amount of antibiotics. He had the mucous membrane from the mouth cavity severely affected and was treated classically without success.

He could not eat any longer since he had foot and mouth disease and severe abscesses on the mucous membrane.

The treatment applied in this case was coloured STOMAPIN and it had excellent results. From the first application the child began eating, the abscesses disappeared and recovery came very soon.

D. A. M. : a 6 years old child. Mouth cavity mucous membrane severely affected. Fever — 39°C. The child had foot and mouth disease which was very painful. After applying coloured STOMAPIN the child could drink, the high fever dropped etc. After the fourth dressing he was completely healed.

K. S. G. : 19 years old. For two weeks he suffered from a severe ulcerous swelling of the mouth cavity, bleeding and pains.

After the first dressing had been applied, the pains calmed down and the condition of the patient improved. After the second dressing the ulcerations disappeared.

M. V. I. : 24 years old. For 10 days he had an ulcerous stomatitis accompanied by a necrotic decomposition on the taste papillae. After applying the first dressing the pains calmed down and the mucous membrane normalized in the next few days.

The biological dressings based on propolis open wide prospects in the struggle with mouth cavity infections.

RESEARCHES ON THE PROTECTING ACTION OF PROPOLIS AND BEE BREAD ON THE INFLUENZA INFECTION

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The works published during the last years reported besides the well-known antimicrobial action of propolis another important antivirus effect of this product (1, 2, 3, 5).

In our experiments we tried to point out this antivirus property or to be more exact the protecting action of propolis and bee bread from virus infections induced experimentally in mice. Although the first results are promising they are still insufficient to draw ultimate conclusions, because the number of experiments was rather small. Further investigations are necessary to determine the optimum dose of active substance, the way and the moment of its administration. The object of our experiments was :

1) to obtain the protection of mice by total intraperitoneal and intranasal administration of propolis and bee bread suspensions before their inoculation with influenza virus (preventive action).

2) the administration of propolis and bee bread 24 hours after influenza infection (the animals show no clinical changes) followed by 3 small daily administrations.

3) to determine the inhibition on viruses in the serum of mice inoculated intraperitoneally with propolis and bee bread suspensions.

Propolis and bee bread were used as antivirus substances. They were dissolved in a 10% dimethyl-sulfoxyde (DMSO) solution (supplied by Schuchard Company).

The data supplied by the specialized literature show that DMSO in concentration of 70% is toxic neither to man nor to animals, it increases the permeability of tissues and is a good carrier for transporting different drugs (4).

One gram of each substance was dissolved in 10 ml DMSO which resulted for propolis in a dark-chestnut solution and for bee bread in a yellow solution with a slight deposit. It was completed until 100 ml by means of a phosphate buffer pH 7.2. The preparation with propolis is an opaque lactescent suspension which deposits with difficulty and the preparation with bee bread is a lactescent more translucent solution which deposits at rest. The suspensions were kept at $+4^{\circ}C$ and before use they were stirred until they became homogenous.

The infection of albino mice was induced by influenza virus APR 8, LD_{50}, by intranasal administration. The mice were 16 to 18 g, $10^{-3.16}$ dilution.

The propolis suspension in proportion of 1 g to 100 ml of 10% DMSO was not toxic to mice, although some experiments with 1 ml suspension inoculated intraperitoneally resulted in a death rate of 30%. Smaller doses were better tolerated by mice. The bee bread suspensions were still better tolerated with no loss in animals even when they were administered in doses of 1 ml intraperitoneally.

Results

The bee bread and propolis suspensions were administered in less concentrated dilutions (1/250) because the 1/100 suspensions brought about the obstruction of the breathing ways of animals. At 1, 3 and 5 days intervals they were administered the influenza virus APR-8 in dilutions of 10 LD_{50} and 100 LD_{50}, respectively.

1) No important differences were noticed between the control animals and those treated with bee bread and propolis suspensions but it should be mentioned that the amount of active substances was low, namely less than 0.5 mg/mouse. The bee bread and propolis suspensions were administered intraperitoneally in doses of 0.2, 0.6 and 1 ml, and at 1 and 7 days intervals, the animals were infected with influenza virus APR-8 in dilutions of 10 LD_{50} and 100 LD_{50}. The highest percentage of survival was recorded with the mice treated with 0.6 ml inoculated intraperitoneally in which the control injection was induced 7 days after the administration of bee bread and propolis (table 1).

Table 1

ACTION OF PROPOLIS AND BEE BREAD SUSPENSIONS BEFORE INFLUENZA INFECTION

Product	Quantity ml	Intervals between inoculations of suspensions and test virus	Survival of non-infected controls %	% of animals surviving the test infection	
				10 LD_{50}	100 LD_{50}
Influenza virus APR-8	3 intranasal drops	—	—	10	0
DMSO (10%)	1	—	100	—	—
DMSO (10%)	1	24 h	—	100	100
Bee bread suspension	0,2	24 h	100	20	—
	1	24 h	100	30	10
	0,6	7 days	100	50	50
Propolis suspension	0.2	24 h	100	10	—
	1	24 h	70	30	0
	0.6	7 days	100	50	30

2) The mice were injected with 10 LD_{50} of influenza virus APR-8. After 24 hours they were inoculated intraperitoneally 3 times every other day with doses of 0.2 and 0.4 ml. Another group of mice were administered 3 times *per os* 0.5 ml of bee bread and propolis suspension. The highest percentage of survival was obtained with the mice treated for 3 days with 0.4 ml suspension inoculated intraperitoneally.

3) One group of mice were inoculated intraperitoneally with 1 ml of bee bread and propolis suspension. Blood samples were taken after 3 days and the serum obtained was tested for virus inhibitors in presence of a virus infection.

Table 2 gives the effect of the same serums inoculated by intranasal and intraperitoneal administration 24 hours after infection of mice with 10 LD_{50} and influenza virus APR-8. The data show clearly that the mice treated for 2 days after their intraperitoneal injection gave the highest percentage of survival.

Table 2

ACTION OF BEE BREAD AND PROPOLIS SUSPENSIONS AFTER INFECTION WITH INFLUENZA VIRUS

Product	Quantity, ml	Administration	% of animals surviving infection with 10 LD_{05}
Influenza virus APR-8	3 intranasal drops	intranasal	20
Bee bread suspension	0.2	intraperitoneal	50
	0.4	intraperitoneal	70
	0.5	per os	40
Propolis suspension	0.2	intraperitoneal	50
	0.4	intraperitoneal	50
	0.5	per os	

Discussion

The 1% propolis and bee bread suspension in 10% DMSO are tolerated well by mice irrespective of the intraperitoneal or oral administration. The intranasal administration is more difficult because of the viscosity of suspensions. It would be possible to try other solvents or to increase the proportion of DMSO. The intraperitoneal administration will result in a higher percentage of survival if the control injection is made at 7 days interval and the percentage will be much lower if mice are infected 1 day after they have received propolis and bee bread suspensions. The mice infected with influenza virus APR-8 (10 LD_{50}) and 1 day later treated for 3 days by intraperitoneal and *per os* administration of bee bread and propolis suspensions give a visibly superior percentage of survival than control animals.

The blood serums coming from the mice inoculated with propolis and bee bread by intraperitoneal administration give a higher percentage of survival in animals infected with 10 LD_{50} of influenza virus APR 8, which makes us assume that these substances could induce virus inhibitors similar to the action mechanism of interferon. To confirm this hypothesis further investigations are necessary which will make these substances utilizable and prove their antivirus effects on both animals and cell cultures.

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ACTION OF PROPOLIS ON THE *"IN VITRO"* HERPES VIRUS

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Foreword

During the last decade more and more investigations were conducted to determine the complex therapeutic action of some hive products.

Among them propolis was the object of numerous experimental and clinical studies centered particularly on its antimicrobial, antimycotic and antiinflammatory properties (2, 3, 4, 10, 11).

The positive results reported by some authors (1, 6) concerning the inhibitory effects of propolis extracts on some infections induced experimentally with vegetal viruses such as mosaic virus, and animal viruses such as influenza virus, opened up the possibility of exploring the actions of propolis on other groups of viruses.

This paper gives some preliminary data obtained by testing the effect of some propolis extracts on the *"in vitro"* experimental infections with *Herpes simplex* virus.

Material and method

A. *Virus.* We used the VR3 strain of *Herpes simplex* virus (HSV) type 1 (5) maintained by successive cultures on simian and rabbit cells.

Cells cultures. The experiment was made on whole human embryo (HE) with the 11—15 *"in vitro"* passage.

Culture medium. The growth medium was IC-65 with 10% calf serum. The culture medium was IC-65 without calf serum.

To test the beach formation beneath agar-agar we used a medium composed as follows : 53% Erle solution, 40% lactalbumin hydrolysate solution, 5% calf serum, 2% glutamine, 200 i.u./ml penicillin, 100 μg/ml streptomycin, 3% agar-agar to 100 ml.

Propolis. The raw propolis was supplied by the Apicultural Plant Complex of Băneasa-Bucharest. Before testing it was brought to such a form as to be tolerated by all cultures (concentration less than 1%).

The toxicity of the product was tested on cell cultures of human embryo by inoculating the culture medium with different propolis dilutions and by maintaining it for 7 days.

The maximum tolerated dose was considered the dilution which caused modifications neither in growth nor in the cell morphology, the dilution used in the experiment being 1/20.

B.1. **Testing the antivirus action of propolis extract with HSV by using extemporaneous mixture**

The culture liquid coming from the cells infected with HSV containing $10^6 ID_{43}$ (infecting dose)/0.1 ml mixed with propolis extract (1/20 dilution) was maintained at 37°C for 24 h, the infecting titration being performed on HE at different intervals.

2. The antivirus action of the product was tested by :

The Method of Comparative Titrations in tubes before and after addition of the product to the culture medium.

To estimate the effect of the reduction of infecting titre (ID_{43}) the cultures were inoculated with decimal dilutions and allowed to absorb for 2 hours; then the culture medium with and without propolis was added and they were cultured for 7 days, during which interval the appearance of the cytopathic effect was observed. The infecting titre was calculated by the Spearman-Kärber method and conveyed in ID_{43}.

The Beach Method with and without addition of the product to agar-agar. The reduction of the infecting titre expressed in terms of units forming beaches (UFB) was estimated by comparison with the values obtained from the control titration and the statistical significance of values was determined by Lorenz tables (8). The cell cultures were put in Povitzki bottles ; after removal of the medium, were washed 2 times with PBS inoculated with 1 ml of virus suspension in decimal dilutions. The groups of dilutions, 4 bottles each, were maintained to absorb for 2 hours, then washed 2 times with PBS and covered with agar-agar including nutritive medium with and without propolis. The

cultures maintained for 6 days at 37°C were covered with neutral red solution 1/10,000 in order to count the beaches. The culture were fixed with 10% formol for 4 hours, then coloured by Giemsa solution.

3. **Estimation of the action of propolis on the increase curve of the infecting titre of HSV in cell cultures**

The cultures were inoculated with about 100 ID_{43} of HSV, allowed to absorb for 2 hours, and washed 2 times with PBS, to which the culture medium with and without propolis was added. The cultures were maintained by this medium for 6, 8, 12, 16, 20 and 24 hours. For each set of experiments, at the specified intervals, the medium with propolis was replaced with the culture medium after 2 washes with PBS and titrations were performed at all these intervals. Further 4 tubes of each series were subject to 2 freezing-defrost cycles then centrifuged at 3000 rpm for 20 minutes. The supernatant thus obtained was titrated on HE cultures. The infecting titres (ID_{43}) were compared to those obtained from control cultures (non-treated with propolis).

4. **Testing the action of propolis extract on the cellular and extra cellular fractions of HSV in cell cultures**

HE cell cultures infected with 10 ID_{43} of HSV with and without propolis were maintained at 37°C for 24 hours ; then the culture medium was removed, thus obtaining the extra cellular fraction of HSV ; the cellular monolayer was washed 2 times with PBS and subject to 2 freezing-defrost cycles, then introduced into IC-65 solution and centrifuged for 20 minutes at 3000 rpm ; the supernatant was the cellular fraction of HSV.

The cellular and extra cellular fractions were used to determine the infecting titres of HSV.

To estimate the dose-response relationship comparative titrations were performed with and without propolis in the medium, in different dilutions, by watching the infecting titre after 48 and 72 hours.

The preservation in time of the antivirus properties of propolis extract was estimated by testing its effects at different intervals after preparation, the solution being kept at +4°C for 3 months.

Results

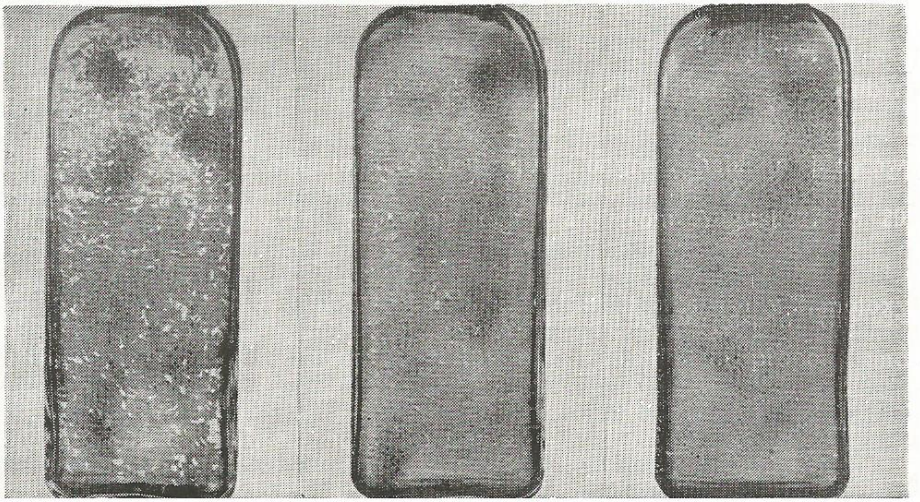
a) **The antivirus action of propolis extract on HSV, under the form of 1st extemporaneous mixture**

Fig. 1. gives the results obtained by testing the antivirus action of propolis extract on HSV. They show a decrease in the infecting titre compared with the control after maintaining it in the mixture for 24 hours.

b) **Influence of propolis extracts on the infecting titre of HSV**

Table 1 presents the results obtained by comparative titrations of the capacity of HSV for infecting HE cell cultures with and without propolis.

Two propolis extracts noted I and II were tested, thus obtaining a significant reduction of the infecting titre $\left(\log \frac{V_0}{V}\right)$ (9).



PROPOLIS

10^{-4} 10^{-5} 10^{-6}

CONTROL

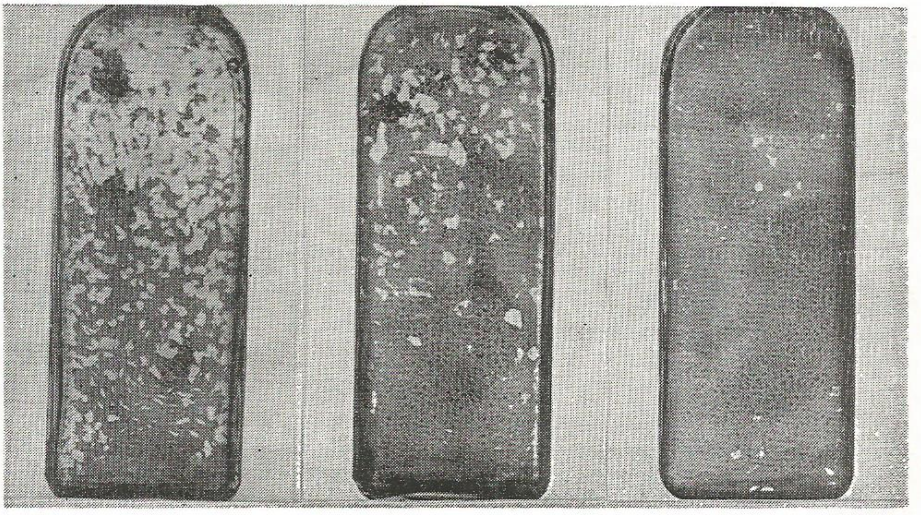


Table 1

EFFECT OF PROPOLIS EXTRACT ON THE INFECTING HSV CAPACITY

	Control titration (Vo)	Control titration (Vo)	Reduction $\left(\log \frac{V_0}{V}\right)$
Propolis extract I	$10^5 DI_{43}$	$10^{1.75} DI_{43}$	3.25
Propolis extract II	$10^6 DI_{43}$	10^2 DI_{43}	4

Table 2

EFFECT OF PROPOLIS EXTRACT ON THE UNITS FORMING BEACHES

	Control titration U.F.B./ml	Propolis extract titration U.F.B./ml	Reduction titration U.F.B./ml
	$2,10^7$	$7,5,10^6$	64%

Table 2 gives the results obtained by the method of reducing beaches beneath agar-agar. The reduction by 64% of the units forming beaches (UFB) is considered according to Lorenz tables as significant, with a threshold of 5% error.

In the beaches obtained in agar-agar in Povitzki bottles with 10^{-4} and 10^{-6} dilutions of HSV in the control and the cultures treated with propolis, one can see the decrease in both the number of beaches and their diameter.

c) **Action of propolis extracts on the increase curve of infecting titre of HSV in cell cultures**

Under the action of propolis maintained in the culture medium for 16, 20 and 24 h the multiplication of HSV takes place at a low level showing a difference from the curve of control (non-treated).

In the set of experiments in which the treatment period of cultures after inoculation with HSV and removal of propolis was shorter, the multiplication cycle is resumed and the differences at the end of experience are no more significant in comparison with the control.

d) When checking up the action of propolis extract on the production of HSV by titrating the infecting ability of cellular and extracellular fractions one can see that 24 hours after inoculation HSV becomes inactive in the culture medium (extracellular fraction) and a decrease in the HSV cell production (cellular fraction) is noticed.

e) **Testing the dose-effect ratio**

Table 3 gives the analysis of relation between the concentration of propolis in the culture medium and its inhibitory effects on the infectant titre of HSV. One can see that beginning with the 1/80 dilution it loses the inhibitory effect.

Table 3

DOSE-EFFECT RATIO

Titration time	M (Vo)	dilution 1/100	dilution 1/80	dilution 1/40	dilution 1/20
48 h	10^5	10^5	$10^{4.5}$	10^4	$10^{3.5}$
Rd *		0	0.50	1.0	1.5
72 h	$10^{5.75}$	$10^{5.75}$	$10^{5.25}$	10^5	$10^{4.25}$
Rd		0	0.50	0.75	1.5

* Reduction expressed in $\left(\log \frac{V_0}{V}\right)$

f) **Testing the ability of propolis of preserving its antivirus properties**

Table 4 includes the results of tests with a propolis extract maintained at +4°C for 3 months. The antivirus properties of the preparation remain unchanged for 1 month. After 3 months a reduction of the properties is noticed. Thus the preparation exhibits a reduced antivirus action demonstrated by its ability to reduce the infecting titre of HSV only after 48 and 72 hours.

Table 4

PRESERVATION OF ANTIVIRUS PROPERTIES OF THE PROPOLIS EXTRACT

Time of testing the extract after its preparation \ Hours since inoculation time	Reduction of infecting titre $\left(\log \frac{V_0}{V}\right)$			
	48 h	72 h	96 h	168 h
7 days	NT	2	NT	3.25
1 month	3	2.5	3.25	3.75
3 months	1.25	1.25	1.75	0.25

Discussion

The results of our experiments show that the propolis extract has an important antivirus action on "in vitro" infection with HSV. The effect of propolis is expressed as a decrease in the infecting titre of HSV by an antivirus action and inhibitory effects on the multiplication of HSV, which is maintained at a low level and restored immediately after removing propolis from the medium.

The antivirus properties shown in the tests with propolis extract during a limited period after its preparation. After a longer period of preservation its inhibitory action on the HSV decreases considerably.

Investigations in progress will establish the mode of action of extract on the infection induced experimentally with HSV and demonstrate whether its inhibitory effects are directly, on the viruses or indirectly on the cellular metabolism.

It should be noted that in interpreting and comparing the data given above the use in these experimental patterns of a natural produce, whose composition is only partially known, calls for certain requirements related to the aspects of standardization of its specific activity, to the testing of its toxic effects on the cellular system, to the preservation of its properties etc.

These difficulties will be overcome by using in the experiment certain fractions (whose composition is known) separated from the crude produce.

USING PROPOLIS IN OPHTHALMOLOGY

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Thanks to its complex composition, propolis (bee glue) possesses a wide range of biological (antibiotical, antiseptic, virulicide, antimycotic, cicatrizing, trophic, anaesthetic, antitumorous) properties already confirmed by numerous research-workers who applied it in therapeutics (HODES et al. 1960, BAVINA, 1960; LAVIE, 1960; BAIXAS et al., 1962; BABIN et al., 1961; MASQUELIER, 1961; A. DEREVICI et al., 1964, 1965, R. CHAUVIN etc.).

Its favourable effects were verified in dermatology, intern medicine, stomatology, O.R.L. etc.

As to its application in ophthalmology no data have been reported as yet probably because there was no appropriate solvent for such product, those existing at present (alcohol, ether etc.) being caustic for local application to the eye ball.

In searching for a compatible solvent tolerated by the eye ball we found an organic amine similar to ethyl diamine which constituted a soluble medium for propolis, thus allowing the preparation of some forms of drugs (eye wash, ointment which can be used in ophthalmology). Since 1971 (N. BAIDAN, N. OIȚA) we have been employing in the ocular therapeutics propolis under the form of a to 5% ophthalmic solution and ointment in concentration of 5 to 10%.

Preparation

a) Preparing eye wash with propolis in 2 stages :

I. Propolis + alcohol 70° = soft extract

II. Soft propolis extract + ethylene diamine + distilled water = ophthalmic solution.

The eye wash with propolis proved to be irritating to eyes producing strong feelings of burning. But its tolerance was much improved by replacing water in formula II with an isotonic macromolecular polymer similar to 10% macrodex. Besides the perfect tolerance macrodex provides also a longer contact of active principles of propolis with the eye and the eye wash is isotonic in contact with the tear liquid.

Given their complex chemical composition the propolis ophthalmic solutions are unstable when associated with some substances in ophthalmology. For instance antibiotics, antiseptics, vasoconstrictors and vitamins make propolis solution incompatible because their pH is below 8, thus making them instable and inactive.

The eye wash with propolis preserved in colored bottles at +4°C proved to be stable for over 30 days.

Its preparation for a longer period was obtained by preparing a dried lyophilized eye wash of propolis. Thus the 2 to 5% propolis eye wash solution used up to the present was lyophilized starting from the

formula : propolis extract + ethylene diamine + lyophilizing excipient + solvent for ophthalmic solution.

The extract of propolis is solved in cool ethylene diamine diluted by 5 ml sterile distilled water. Then 60 more ml water are added and completed up to 100 ml with the solution necessary to lyophilisation. It is filtered under aseptic conditions and poured into bottles of 5 ml similar to those used for antibiotics, after which it is lyophilized and bottles are stapled under inert gas. Ethylene diamine plays the role of a solvent for the propolis extract, the active principles are soluble in proportion of 98 to 100%, which cannot be obtained with the known solvents tolerated by the eye. The ophthalmic solution is prepared extemporaneously with 10% macrodex. The dry eye wash can be preserved for at least 1 year.

b) The preparation of the ophthalmic ointment with propolis raises the same technical problems because it is insoluble in water. By dissolving it by means of the same amine used to prepare the eye wash and by incorporating it with a basic ointment (eucerine) we could obtain an active ointment which is well tolerated by the mucous ocular membrane : propolis soft extract + excipient.

Therapeutic results

Since 1971 we have been using in the ocular therapeutics a 2 to 5% ophthalmic solution and a 5 to 10% propolis ointment. These drugs were adopted by the current practice instead of the classical preparations which proved to be insatisfactory or inferiour to propolis with special regard to its biological effects (antiseptic, antibiotic, antivirus, cicatrizing and locally trophic).

Thus, remarkable results were obtained with the eye wash and ointment with propolis in a number of various ocular affections :

— The cornea conjunctive tissue burns including the chemical ones (with quicklime, aniline etc.) treated from the very beginning with frequent propolis instillations and subpalpebral ointment applications were rapidly cured completely or with minimum sequelae. The palpebral burns or those affecting the neighbouring teguments were also cured in a shorter period in comparison with classical treatments.

— Favourable effects were noticed in various affections of the ocular annexes (blepharitis, conjunctivitis with different etiologies, eczemas of eyelids, plagues), which were cured or visibly improved.

— In most affections of fore pole, particularly in microbial or virus keratites, cornean ulcers, propolis gave spectacular results.

Since 1972 numerous cases of epidemic lingering keratoconjunctivitis with adeno-virus etiology, stubborn to any classical therapeutics have appeared on the territory of Moldavia. We selected 15 hospitalized patients with serious K.C.E. who were treated with such preparations with good results, being cured in a short period (N. BAIDAN, N. OIȚA, 1975). It should be noted that the patients with K.C.E.

were not separated from the others with medical or surgical affections. Nevertheless no case of intercontagion or iatroinfection was reported because the prophylactic propolisotherapy was applied to all of them. In 6 patients the therapy with propolis resulted in the cure of disease in the conjunctival stage thus avoiding the stage of cornean superficial dotted keratitis with a lingering healing.

The preparations with propolis were also used with good results in the pre-operative stage in order to provide asepsis of the eye ball, as well as post-operatively in order to avoid infection and accelerate the healing process.

Conclusions

1. Thanks to its complex chemical composition propolis has a wide range of biological properties useful to the medical therapeutics (antimicrobial, cicatrizing, antiseptic, antimycotic, antivirus, anaesthetic, trophic properties); it was possible to use it in the ocular therapeutics after discovering an appropriate solvent tolerated by the eye — ethylene diamine.

2. Ophthalmic solutions with propolis (2 to 5%) and propolis ointment (5 to 10%) were used with excellent results in treating ocular burns and traumatisms and their annexes, microbial and virus inflammatory affections of the fore pole of the eye and their ocular annexes in order to obtain a pre- and post operative asepsis of the eye ball. The ophthalmic solution got considerably more tolerable by incorporating 10% macrodex in it as an excipient in place of distilled water.

3. Propolis conditioned as a dry (lyophilized) eye wash can be preserved for a long period (at least 1 year), and used in case of need by adding to it 10% macrodex.

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THE TREATMENT WITH PROPOLIS OF NONSPECIFIC ENDOBRONCHITIS

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The scientific research of the therapeutic qualities of propolis began only 10—15 years ago.

Beginning in 1964 we paid a lot of attention to the patients hospitalized but who proved to have the wrong diagnosis of lung tuberculosis instead of pneumonia. In most of the cases, the cause had been the inefficient treatment of pneumonia. According to the evolution of the disease we distinguished 3 groups of pneumonia : prolonged pneumonia, chronic pneumonia fits, pneumonia with evolution without symptoms or with few ones (diagnosed when the prophylactic exam was performed or when finding other diseases, too).

Between 1964—1969 we analysed the data concerning 76 children between the age of 2—14 years old in whom the nonspecific pneumonia was complicated with bronchic affections.

According to former diagnoses the patients were grouped as follows : primary complex (51), nodule lung tuberculosis (8), Grancher spleen pneumonia (6), infiltrative lung tuberculosis (11), bronchoadenitis with exudative dry pleuresy (1).

After examining these children we established the following diagnoses : endotorax lymphatic ganglions tuberculosis in the calcification stage ; chronic pneumonia I—II fits — 37 children, chronic pneumonia at the IInd fit (11) ; endotorax lymphatic ganglions tuberculosis in the calcification stage (multiple calcification) ; chronic pneumonia II—III fits ; ganglion tuberculosis in the induration stage ; prolonged br nchopneumonia (12) ; tuberculin response curve ; post vaccinum allergy ; chronic bronch pneumonia : staphylococcis pneumonia (2) ; abscessed pneumonia (1) ; chronic tuberculous intoxication (1) ; pneumonia with small focuses (1).

All children sick of prolonged pneumonia and of chronic pneumonia fits were treated — before hospitalization — with antibiotics and sulphonamides. Part of them were also treated with γ-globulins, and physiotherapy. However these did not give efficient results and the children were transferred to the T.B. hospital.

Almost all patients had cough, wheezing from lungs, spittle. In 22 of the children the sedimentation speed was very high, in 37 it was medium and in 17 it was irrelevant or normal. 12 patients had the white cells increased up to 20,000/mm^3, 25 had 15—20,000 and 18 had over 10,000.

21 patients had white cells within normal limits. 3 children had leucopenias, and 57 deviations of the white cells formula. 18 children had traces of albumin in their urine.

The biochemical blood examination indicated some deviations from the normal standard.

Thus in 14 patients the thymol response was between 6—10 units, between 10 and 24 units in 3 patients and within normal limits in the rest of the patients.

The quantitative rations of albumins and globulins did not indicate essential deviations but the globulin fractions have modified especially because of γ-globulins. In 23 children the content of γ-globulins varied between 24 and 26% and in 31 children between 21 and 24.

The Roentgen examination indicated great variations. In all children the curve of the lungs was modified in this exam.

Most often the drawing was macro-trabecular and more seldom areolar or reticular. There was sometimes even emphysema in some segments or on a small area of the lung. There were also observed small focuses and shades of medium size.

In some of the children cavities in lungs were found.

In the first days of hospitalization the patients were prescribed antibiotics, vitamins, γ-globulins, fractioned transfusion, ultra-short rays, ionophoresis with calcium etc.

The children with cough and wheezing even after this treatment were prescribed bronchoscopy.

The bronchologic examination was performed under intravenous narcosis accompanied by barbiturates. For bronchoscopy the "Friede" apparatus was used. Almost in all children the endobronchitis was catarrhal and in the bronchia lumens a mucous content was found.

Only in 3 children, the bronchia mucous atrophy was observed. On its surface mucous strata stuck. In these children, the removal of the mucous from bronchias ensures disappearance or reducing of the cough for a period of 5—8 days.

In a series of patients (with chronic pneumonia II—III) a large oedem of the lobular mucous and the filling with mucous of the lobular bronchia and bronchioles was observed. During the bronchoscopy some content from the bronchia was taken for insemination for secondary microflora and for the tuberculosis bacilli. From the secondary microflora I isolated golden haemolytic staphylococcus (49 patients), streptococcus (14) and catarrhal micrococcus (5).

The resistance to some antibiotics of the isolated strains (of staphylococci and streptococci) was determined (see table).

Antibiotics	Growth inhibition zones (mm)				
	0	1—10	11—15	16—20	25
Penicillin	43	22	3	—	—
Streptomycin	18	21	20	5	4
Tetracyclin	11	27	17	10	3
Oleandomycin	8	10	20	22	8
Erithromycin	10	14	15	17	12
Neomycin	6	11	19	15	17
Levomycetin	4	6	9	21	28
Canamycin	—	—	—	5	13

The patients were divided into 3 groups.

In the first group were 21 patients. They were administered antibiotics to which the isolated strains were sensitive. The antibiotics were administered internally, in the muscles intravenously or in the lungs and also in aerosols.

The second group included 32 children who were administered antibiotics sensitive to isolated cocci strains and a 10% watery propolis solution — in the form of aerosols.

The aerosols were made 4—6—8 weeks. The antibiotics were administered for 10—20 days, alternatively, every 5—7 days.

The third group had 23 children, especially those with antibiotics resistant microflora. They received 10% propolis watery solution in aerosols and 30% alcohol propolis solution — 15 up to 35 drops, 3 times a day, one hour before meals.

For aerosols, the portable PAI-1 or PAI-2 apparatus was used.

The amount of propolis solution used depended on the age of the child.

Each sitting of aerosols lasted 10—15 minutes.

The best results were obtained in the children from the second group. The treatment of non-specific endobronchitis lasted 4—6 weeks and in the bronchias content there was no secondary microflora.

In the children of the first group the average duration of the treatment in non-specific endobronchitis ranged between 8 and 12 weeks.

In the children from the third group the average duration of the treatment ranged between 10—16 weeks. Some of them, when examined bacteriologically, proved to be sensitive to antibiotics, penicillin included. Propolis should be considered an additional means of treatment of the non-specific endobronchitis in the children sick of non-specific pneumonia.

PROPHYLAXIS AND TREATMENT OF THE NONSPECIFIC CHRONIC PNEUMONIA AND BRONCHIC ASTHM IN CHILDREN

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There are often found in children some pathologic conditions of lung among which chronic pneumonias are prevailing, even in the children who had never been subject to accute pneumonia.

In the anamnesis of these children, accute catarrhs of the upper respiratory tracts are often found : these children have especially adenoïd vegetations. They have often and almost all the time chronic rhinopharyngian swelling focuses.

The aggravation of these infection focuses in the palate and rhinopharyngian tonsils is manifest by symptoms which present catarrhs of the upper respiratory tracts, anginas, bronchites and even pneumonias. Besides, chronic amigdalitis brings about heart and kidneys diseases.

The pediatrics ambulatory near the hospital nr. 4 in Kiev used in 1969 the 5% propolis-alcohol solution proposed by Dr. A. N. PESCHANSKI, under the form of electroaerosoles in 52 children who had had frequent catarrhs of the upper respiratory tracts, bronchites, chronic pneumonias, bronchic asthm. The positive effect was stable.

The solution is prepared from propolis extract in 96% ethylic alcohol. It contains a complex mixture of organic substances (resins, balms, polioxyflavones, etheric oils, vitamins and minor elements).

Before having been used, the solution was researched in the department of microbiology of the Medicine Institute in Kiev (research assistant V. I. POCHINOK). There was established that the solution has antibacterial properties, inhibiting — in 1 : 4 dilution — the growth of the microorganisms (the solution has a more active effect on the grammepositive microflora as compared to the grammenegative one).

In the research Institute for tuberculosis and thorax surgery "F. G. Ianovski", trials for the bactericidal effect of the solution were performed. The trials were done with a pathogenic staphylococcus strain nr. 209.

The experiments proved that in a 1 : 8 dilution the 5% propolis alcoholic solution inhibits the growth of the staphylococcus (tests done by dr. S. P. SNISARENKO).

For inhalations, the propolis alcoholic solution was used, emulsified in peach oil, apricot oil and eglantine oil in dilutions of 1 : 3, 1 : 2, 1 : 1 or in the same dilutions in distilled water. The children with asthm were prescribed the dilution 1 : 2.

When they found fungi — *Candida* — (from medicines) in the mucus smear from the pharynx vestibule or from spittle, the 1 : 1 dilution was administered.

The propolis emulsion is poured into a spray connected to the lightening mains. The fine emulsion particles mixed with air and electrically charged, are deposited on the pathologic areas of the upper or lower respiratory tracts covering the bronchic tree and penetrating it.

In 1969 the treatment with aerosoles was applied in 52 children between 2 and 14 years old, from among which 15 suffered from chronic pneumonia, 11 from upper respiratory tracts catarrh, 10 from bronchic asthm, 5 with chronic pneumonia, 7 with catarrhal bronchitis, 2 with tracheitis, 1 with pharyngitis and 1 recovered after a pneumonia.

Before beginning the treatment, the children were tested for sensitivity to antibiotics. Then, they were prescribed 5—20 sittings of aerosoles with propolis alcoholic solution. The children with affections of the upper respiratory tracts breathed in the nose and those with affections of the lower respiratory tracts breathed in the mouth. The duration of the sittings was 1—3—5 minutes, and then a rest for 20 minutes.

After the treatment, in 14 pacients with chronic pneumonia, the catarrhal phenomena were got rid of; in 10 children sick of bronchic asthm, the crises were strangled and only 1 fell sick again, after an influenza. By treating the catarrhs, pharyngites, tracheites, all 25 children were cured.

PROPOLIS IN COMPLEX TREATMENT OF PULMONARY TUBERCULOSIS

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During the International Apicultural Congress held in Moscow, August 27 to September 2, 1971, a Symposium on use of bee products in human and veterinary medicine took place from August 27 to 29.

The papers delivered at the Symposium were published by the APIMONDIA Publishing House. This paper is one of them.

The positive results obtained by us (Z. H. KARIMOVA, K. I. SEVASTIANOVA, and L. M. VAINER, 1960) and by other authors (I. M. RABINOVICH, 1960 ; V. P. KIVALKINA, 1964 ; Z. G. CHEANYSHEV, 1960) with respect to the antimicrobial effect of propolis on various types of microbacteria of tuberculosis urged us to test the effect of propolis on group of hospitalised and out-patients.

The tests were made on 147, six to fifty years old patients : 109 with broncho-pulmonary tuberculosis, 15 — tubercular adenopathy, 11 — tracheo-bronchitis adenopathy, and 12 — with kidney tuberculosis. Patients were treated either at the tuberculosis hospital (40) or as out-patients (107).

The following forms of pulmonary tuberculosis were recorded : fibro-cavitary (50 patients, in 8 also tuberculosis of the bronchi), miliary disseminated — haematogen (40 patients, two with tuberculosis of the bronchi), noduled tuberculosis (10), Grancher splenopneumonia (11), and broncho-kymphodenites. In the sputum of all patients, microbacteria of tuberculosis (Koch bacillus) were found.

Treatment with propolis was applied in particular to patients in advanced stages of pulmonary tuberculosis. The classical medicines used previously in these cases either had not had the expected result, or the patients could not tolerate them because of a high sensitivity to them.

The dose of propolis was administered 3 times daily, before meals. To the first group (73 patients) propolis oil was given ; to the second (20) — water-alcoholic propolis solution, to the third (32) — propolis prepared with butter and tuberculostatic medicines, and to the fourth — tuberculostatic medicines and butter without propolis. According to the stage and form of the affection, the treatment lasted for 4 to 10 months.

Of the 107 out-patients, 101 were treated with propolis alone ; 6 were also administered tuberculostatic medicines during the pauses in between propolis treatment.

Of the same 107 out-patients, 53 had been previously treated with a great quantity of tuberculostatic medicines with no effect, while 12 were not treated as individual counterindications existed.

In 50 patients with fibro-cavitary tuberculosis — 30 being treated with 15% propolis oil and 20 with 20% water-alcohol solution — the general condition improved : temperature and sedimentary speed de-

creased, haemoptysis, cough, expectoration and thorax pains disappeared, their appetite restored, and their sleep was normal again.

In 19 patients treated with propolis oil cavities healed in the 4^{th}—10^{th} month of treatment.

Of 20 patients to whom propolis alcoholic solution was administered, cavities only healed in two. In the others they diminished substantially, the nodular and infiltra thickenings around the cavity resorbed, all symptoms of tubercular intoxication disappeared, and the general condition of the patients has improved allowing for surgical operation.

Of 40 patients with miliary disseminated pulmonary tuberculosis, 27 were administered propolis oil and 13 alcoholic solution. In 33 the dissemination nodules have substantially diminished, whilst in 7 the general condition remained the same.

In 21 patients with noduled tuberculosis and Grancher splenopneumonia, together with the improvement of the general condition also a regression of the tubercular process was recorded. 5 patients were administered both propolis oil and tuberculostatic medicines, and propolis is supposed to have accelerated healing of lesions.

All 6—20 years old out-patients (26) with broncho- and lymphoadenites were administered 15% propolis oil. In addition, to two patients with fistules also propolis with chemically pure vaseline was applied. Positive results were obtained in all patients.

In 12 patients with cavity tuberculosis of kidneys no positive result was obtained after a long treatment with tuberculostatic medicines. To 5 of them surgical operation was suggested but they have not accepted. The treatment with propolis oil healed them clinically.

Since April 1968, intratracheal application has been practised of 10% aqueous extract of propolis in tubercular patients with specific lesions of bronchi revealed by bronchoscopy.

The aqueous propolis extract was preparated as follows : in a refractory glass retort 100 ml distilled water were poured, and 10 g top-quality thinly cut propolis which had been extracted in a water bath at 100°C for one hour, and the mixture was stirred continuously. One hour later, the mixture was filtered through a thin layer of cotton, and poured through a funnel into a glass pot. When ready, the extract has a dim light-brown colour. It is kept in refrigerator at 40°C.

By bronchoscopy, infiltrative tuberculosis in progress of the main bronchus to the right side was recorded in 4 patients, the same process to the left — in 2 patients, infiltration-ulcer tuberculosis in progress of the bronchus of the lower lobe to the right side — in 6 patients, and infiltrative tuberculosis in progress of the mucous spur — in two patients.

The extract was administered every two days, with previous tuberculostatic treatment having been applied. After anaesthesia of the larynx with 1% dicaine solution, 5 ml aqueous propolis extract was administered — 25—38 times. In 5 patients, after 15 doses, treatment was continued with aqueous propolis extract under the form of aerosols.

Healing per primam intentionem of specific affections of bronchi was recorded in all patients, in 2—3 months, much sooner than with tuberculostatic medicines (streptomycin or tubazide). One of the patients had had permanent haemoptysis for two years. Here are several data of his disease record :

Patient M., 32 years old, suffering of fibro-cavity tuberculosis since 1958 was treated in hospital several times with a great quantity of tuberculostatic medicines. Late in 1966, slight blood spitting started ; haemoptysis has then intensified periodically. Haemostatic means were not efficient. Starting April 1968, 10% aqueous propolis solution has been administered intratracheally. After 10 doses, haemoptysis stopped and never occured again.

The preliminary results obtained in treatment with 10% aqueous propolis extract of pulmonary tuberculosis with tuberculosis involvement in bronchi allows us to appreciate its effect as positive.

Propolis is an efficient additional substance in the complex treatment of patients with pulmonary, bronchial and kidney tuberculosis and of lymphatic glands. It removes a number of toxic symptoms of tuberculosis, and facilitates resorption of infiltrating and nodular thickenings. Also, after treatment with propolis extract, no Koch bacillus existed in sputum any more.

When tuberculostatic medicines are not tolerated and when the pathogen agent is resistant to them, treatment with propolis alone improves the general condition of patients.

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PROPOLIS EXTRACT

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For obtaining propolis extract, high-quality propolis is broken into pieces, the visible mechanical impurities are removed, and 96% rectified alcohol is added (100 g alcohol for 10 g propolis). Extraction takes place for two days at room temperature. First, the substances are thoroughly mixed for 30 minutes, and subsequently the recipient is stirred from time to time.

After decantation, the colour of the alcohol may range between light yellow and dark brown, according to the quality of propolis and concentration of the solution. On the third day, the mixture is strained through one or two sheets of gauze. The extract obtained is concentrated by evaporation (to 2/3), then is separated by distillation in water bath

until a brown, viscous mass, with pleasant odour, is obtained. The propolis extract is kept in covered recipients, under usual room conditions.

Propolis extract is used for therapeutical external application under the form of 10, 20 or 30% ointment prepared with melted anhydrous lanoline, butter, vaseline etc.

At the clinic No. 22 in Kiev, A. CHUPRINA, head of the obstetrics and gynaecology section, and physician G. I. CHUMACHENKO have successfully used the 30% propolis ointment prepared with anhydrous lanoline in treatment of obstinate erosions of uterus cervix.

The 42 years old patient K. had an obstinate erosion of uterus cervix. During the classical treatment (1966—1968) she underwent two biopsies and then electrocoagulation.

In 1970 the erosion was not yet healed, having the size of 2×2.5 cm, which demanded amputation of uterus cervix. A. Chuprina and G. I. Chumachenko decided to treat her with propolis ointment prepared with anhydrous lanoline. Plugs with ointment were introduced 7 times, every two days. The erosion has healed completely.

This confirms the fact that propolis is efficient in the treatment of erosions of uterus cervix and of other hardly healing wounds. The preparation is antimicrobial and analgesic, and stimulates granulation and epithelization.

EXPERIMENTAL AND CLINICAL RESULTS OF TREATMENT OF ACUTE AND CHRONIC COLITIS WITH PROPOLIS

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Investigations were undertaken for determining the efficiency of propolis in treatment of acute and chronic colitis, as well as for precisely establishing its effects. We made preliminary tests in vitro of the isolated intestine of a guinea pig, by Magnus method. We found that an aqueous solution of propolis — 1 : 2000 to 1 : 10,000 —, intensifies intestinal contraction and accelerates the tonus. When atropine is added — 1 : 100,000 —, tonus slows down and the frequency of contractions becomes normal.

Also, after administration of propolis, the effect of 1 : 100,000 papaverine declined.

Under the action of propolis, the effect of acetylcholine on the isolated intestine intensifies.

These experimental results show that propolis has an effect on the vegetative nervous system — in particular on the M-cholinergenic structures entailing changes in the peristalsis of the intestine.

Clinic observations were made of 45 patients (15 suffering of subacute colitis, and 30 of chronic colitis). 30 were females, and 15 males, aged between 20 and 65, most of them being 45 years old. The affection had set in 3 months to 10—15 years ago. The main criteria in choosing the patients were typical anamnesis data and concerning the objective condition, as well as X-ray photograph confirming colitis.

The propolis collected in 1971 in three regions in north-west Bulgaria was administered *per os*, as alcohol extract, in 30% water dilution.

The extract was obtained by maceration of propolis in 96% ethyl alcohol, 1 : 5, at room temperature, for 48 hours. Because of the zone where it had been collected, the extract of propolis had an intense red colour, and reacted to acid (pH 3.2—4.0).

At the beginning, 30 drops of alcohol propolis solution — diluted in a glass of warm water or unboiled milk were administered three times a day one hour before meals, in order to check the reaction of the body. Then, 40 drops were administered, three times a day, before meals. Propolis was administered irrespective of diet No. 4 (according to Pewzner). The subjective symptoms — condition of the patient during treatment, defecation, meteorism etc. were recorded, as well as objective symptoms — changes in the aspect of the tongue, in weight, and after treatment, in each patient.

The fundamental clinic criteria were radioscopy, irrigoscopy, and radiography of intestines. Also rectoscopy was made to all patients. During and after treatment, coprological, bacteriological and serum (Vidal test) tests were made.

Results

In all 45 patients treated with propolis results were positive — very good in 26, good in 12, and satisfactory in 5. Only in 2 patients no improvement, was recorded. Pain diminished starting in most cases on the 7th day of treatment, disappearing completely on the 19—20th day. Also a positive effect was recorded on constipation. In most cases, defecation improved after 5—10 days of treatment. Only in 4 patients no improvement was recorded. In all patients the sensation of pressure in the abdomen and phenomena of meteorism disappeared after the 5th day of treatment. The sleep of 17 patients improved, and in 13 the symptoms of neurasthenia disappeared. Also, in most cases of hypertension (34 patients) the treatment had a hypotensive effect. Blood pressure decreased with 10—15 mm barometric column — systolic pressure, and 5—10 mm — diastolic pressure.

In 5 cases, hypotonis occurred. The best results were recorded in hypertonic patients : substantial improvement of objective parametres and of subjective ailments.

The results obtained justify our recommendation to use propolis for treatment of both colitis and hypertonia.

Objective criteria

The weight of 9 patients has increased moderately. The aspect of the tongue became normal in all patients, and the condition of the abdomen improved (the pain when palping the large intestine disappeared in 36 cases, as well as sigmoid spastic pains).

By X-ray examination the following results were recorded: before the treatment most patients had spastic colitis, and 5 ulcerated colitis. After the treatment, the condition of the mucous membrane was unchanged, but the results obtained can be considered as positive because a tendency of normalisation of functional phenomena correlated with the improvement of the subjective condition of the patients was recorded. In 15 patients a residual liquid was identified before the treatment (a hypersecretion of the small intestine); after the treatment, the liquid only existed in 3 patients.

When examined previously to the treatment, in 21 patients profound segmentary peristalsis was recorded; after the treatment, the peristalsis was normal. In 12 patients, very marked spastic dyskinesis and increased antiperistalsis in the small intestine were recorded before the treatment. In all cases, these phenomena disappeared after the treatment.

All patients complained of pain when palped; by X-ray photography the pain was located in the zone of the duodenal bulb, the descending colon and of the sigmoid colon. In most cases the pain disappeared after the treatment. The excitation of the mucous membrane disappeared in most cases, which shows melioration.

The X-ray examination of the rectum of all patients revealed a non-specific and superficial, sub-acute or chronic inflammation of the protosigmoid — in 32 cases, and a post-disinthery chronic protocolitis — in 7 patients. In all patients, functional changes of the rectum were found, especially hypertonia (spastic, peristaltic, or rigid), the latter — in 30 patients. Hypotonia with atony was recorded in 15 patients. Examination of the patients after the treatment revealed a substantial improvement of the rectal spasm, with hypertonia and normotonia in 22 patients. In 12 patients, hypotonia and atony were found, and no changes were recorded in 4 patients.

The bacteriological tests of faeces revealed pathogenic flora in 28 cases — caused by stasis and secondary infection. Staphylococci, streptococci, Proteus, and *Escherichia coli* were isolated. The tests made after the treatment showed that *Escherichia coli* and *Proteus* existed only in 8 patients; the faeces of 20 patients were sterile. The antimicrobial effect of propolis is obvious — the strongest on staphylococci, gradually decreasing on streptococci and proteus, with the weakest — on *Escherichia coli*.

Coprological tests indicated steatorrhea in 12 patients. After the treatment, no significant digestive disturbance was recorded.

Sampling of urine and blood, the tests of flocculation, and the protein tests did not reveal substantial variations as against the normal

average. The treatment with propolis has caused neither allergic nor toxic phenomena.

Conclusions

1. The effect of the propolis aqueous solution was studied on 45 patients with subacute or chronic colitis.

2. The effect of propolis was found to be positive : very good in 25 patients, good in 12 patients (by considering subjective and objective data).

3. Propolis has a favourable effect in patients with constipation syndrome.

4. Propolis was found to have a hypotensive effect.

5. The positive effect of propolis on the activity of intestines (peristalsis and tonus) is conditioned by its action on the M-cholinergenic system.

6. The bactericidal effect of propolis on streptococci, staphylococci, proteus, and *Escherichia coli* existing in patients intestines was proved.

7. The doses of propolis administered were tolerated by the patients, no toxic phenomenon being recorded.

RESULTS OF USE OF PROPOLIS IN GYNAECOLOGY

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Considering the antibacterial and regenerating effect of propolis, we used propolis extract in gynaecological affections.

The above mentioned effects — known for long —, were also confirmed by the experiments made by us.

Material and method

Two groups of patients were treated with propolis.

The first group included 35 patients with various post-surgical gynaecological affections : atonic vaginal wounds following fluor vaginalis perineoraphae, inflammations of uterus and other pathological modifications in Portio vaginalis — except cancer.

The second group included 60 patients in whom alongside with modifications in Portio vaginalis, also *Trichomonas vaginalis* was identified.

3% and 15% propolis solutions of 96% ethyl alcohol were used, prepared as follows : propolis was extracted (4 days) in 10 volumetric units of 96% ethyl alcohol, at 37°C. After filtration, the solution obtained was used for preparation of 3% and 15% propolis solutions.

The antimicrobial activity of this extract was ascertained by the fact that it inhibited the growth of *Staphylococcus pyogenes,* Oxford 209 P strain. The inhibiting activity was determined for 1 ml/600 g live weight.

The patients in the first group used the 3% and 15% extracts alternately, painting the affected zones daily. The treatment lasted for 12—18 days.

The second group was divided in three sub-groups :

To the 25 patients in the first sub-group, Metronidazol 0.25 was administered per os twice a day, and Metronidazol 0.5 intravaginally once a day, for 10 days.

The 18 patients in the second sub-group underwent the same treatment as the patients in the first sub-group — for 10 days, and in addition Moor plugs were daily introduced, intravaginally.

The 17 patients in the third sub-groups were also treated with Metronidazol and Moor plugs, and in addition daily paintings with propolis extract were made. 10—15 plugs were used, and 10—15 paintings with propolis extract were made to every patient.

Results

Table 1

RESULTS OF TREATMENT WITH PROPOLIS IN THE FIRST GROUP OF PATIENTS

Diagnosis	Nr. of patients	Results		
		+	±	—
Cervix erosions	10	10	0	0
Vegetations, leucorrhoea	10	6	4	0
Post-surgical sores	8	8	0	0
Vaginitis	8	4	2	0

As shown in table 1, propolis speeded up the healing of hardly healing vaginal sores and of post-surgical vaginal sores.

After the treatment with propolis, the white secretion would become physiological, and an essential improvement in the subjective condition of the patients was recorded.

Table 2 illustrates the results of the treatment of the patients of the second group.

Table 2

VARIOUS KINDS OF TREATMENT OF VAGINITIS AND *TRICHOMONAS*

Treatment	Nr. of cases	Results		
		+	±	—
Metronidazol	25	2	23	—
Metronidazol and Moor plugs	18	6	12	—
Metronidazol, Moor plugs, and propolis	17	15	2	—

After healing from *Trichomonas*, an improvement of the condition of uterus cervix was recorded when propolis was used. The inflammatory processes in endocervix and in vagina have disappeared. The vaginal secretion became normal in several days. Only in one case of treatment with propolis moniliasis set in. We considered it as a complication and cured it with antimycotic medicines (nistatin).

We also mention that we also used propolis in breast cancer. On the post-surgical wound (when it was open, moist and necrotized) we spread 3% propolis solution. Because of the wound, the breast had swollen, was hard and ached. As soon as the third day of treatment, the wound was dry and epithelization started.

Conclusions

1. The propolis extracts have speeded up healing of hardly healing wounds following gynaecological surgical operations.
2. In the patients in whom Trichomonas had also to be cured and who tolerated treatment with propolis too, the secretions became normal sooner and in more of them, than in patients to whom propolis has not been administered.
3. Secondary effects of propolis (moniliasis) occurred only in one case (1.1%), being cured with nistatin.

EXPERIMENTS FOR TREATMENT WITH PROPOLIS IN INFLAMMATIONS OF VAGINA AND UTERUS CERVIX

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The paper dwells on the results of the experiments conducted by the authors with propolis extracts used in treatment of inflammations of vagina and uterus cervix.

Material and Method

The treatment was applied to 90 patients — 16—62 year old — with inflammation of the vagina and uterus cervix. Three groups were formed :

The first included 47 patients in whom the inflammation was caused by *Trichomonas vaginalis.*

The second group — the patients in whom the inflammation was caused by pathogen fungi.

The third group — 15 patients in whom the inflammation was due to an infection of mixed bacterial origin. Pathogen staphylococci and streptococci were found.

3% propolis solution — in 96% ethyl alcohol — was used. The antibacterial activity of the ethyl alcohol fraction was of 3 mg/ml nutritive medium, tested on *Staphylococcus pyogenes* (strain Oxford 209 P).

In all cases treatment lasted for 7—10 days, being applied once daily.

Results

The results obtained are given in tables 1 and 2.

Table 1

RESULTS OF THE TREATMENT APPLIED IN INFLAMMATIONS CAUSED BY *TRICHOMONAS VAGINALIS*, PATHOGEN FUNGI, AND VARIOUS BACTERIA

Diagnosis	Nr. of cases	Treatment applied			
		Propolis		Sulfadovaginol. Vagosan	
		Healed	Not healed	Healed	Not healed
Group I Erosion of uterus cervix + Trichomoniasis	47	19	5	13	10
Group II Erosion of uterus cervix + Moniliasis	28	11	3	9	5
Group III Erosion of uterus cervix + Bacterial infection	15	7	1	4	3
Total	90	37	9	26	18

Table 1 shows that the best results were obtained in the third group (mixed infection caused by at least two kinds of microorganisms). In 7 patients treated with propolis inflammations were healed, and an obviously improved condition of the uterus cervix was also recorded.

Our previous investigations revealed the fact that staphylococci and streptococci are highly sensitive to propolis.

Also successful were the treatments in the second group (trichomoniasis), 18 of the 24 patients being cured.

In the group with inflammation caused by a pathogen fungus, only in 11 patients of the 24 investigated improvement was recorded.

The number of healings in the three groups of patients treated with propolis is greater than that in the control group.

Results obtained in the control group

Table 2

RESULTS OF THE TREATMENT OF EROSION AND INFLAMMATION OF VAGINA

Treatment applied	Nr. of patients	Healed		Not Healed	
		Colpitis	Erosion	Colpitis	Erosion
Propolis	46	37 (80.4%)	18 (39.2%)	9 (19.6%)	28 (60.9%)
Sulfadovaginol and lavage with Vagosan	44	26 (59 %)	9 (20.7%)	18 (41 %)	35 (79.6%)

Table 2 includes compared results. Of the 46 patients treated with propolis, 80.4% healed, and in 39.2% even erosion healed or substantial improvement was recorded.

In the control group to whom other medicines were administered, the percentage of healings was lower (59% inflammation of vagina, and 20.7% erosion).

During the treatment with propolis, allergy phenomena were recorded in 5 patients inflammation, rash, and congestion of the vulva. These symptoms disappeared after administration of anti-allergic medicines and when propolis therapy was over.

Conclusions

1. Propolis extract proved efficient in treatment of inflammations of vagina and uterus cervix caused by *Trichomonas vaginalis*, pathogen fungi, and mixed bacterial infections.

2. Propolis extracts gave the best results when administered for 7—10 days. Longer treatment can cause, in a few cases, reactions of allergic nature.

PROPOLISOTHERAPY IN BRONCHIAL ASTHMA

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Foreword

Bronchial asthma is a disease of respiratory tract encountered in all zones and groups of population, which causes much sufferings and long lasting disablement.

It has various pathogenic causes and consists of repeated crises of respiratory dyspnaea (difficulties in breathing with the chest filled with air because of constriction of bronchia). No radical treatment has been found for this disease up to the present. Different symptomatic and bronchodilator medicines are used to suppress crises and other etiopathogenic drugs to prevent them.

The application of corticotherapy offers a means of mastering disease but it causes adverse, iatrogen effects and dependence on drug (4, 17). As we noted in our previous clinical experiments these crises disappeared or became less frequent when applying propolis to treat some endocrinopathies (10, 11), so we decided to check the action of propolis on bronchial asthma. This bee product seems to be effective in both stopping crises by its antiinflammatory, anaesthetic and locally eutrophic effects (1, 2, 3, 9, 12, 13, 16, 17) and in preventing them by its normal and immunoprotective effects (11, 12) which increase the protective function of the body.

Material and method

Patients : 30 voluntary patients of both sexes differing in age and profession but suffering from the same endocrinopathy (spasmophilia and benign hypothyroidism) and the same simple bronchial asthma were distributed in 6 equal groups. Each group was given a different treatment but all groups were examined weekly by the same physician who checked the same parameters and used the same complex therapeutic control card. Diagnosis, simple bronchial asthma, was previously determined by a respiratory tract disease service. The treatment lasted 30 days after which only positive cases were re-examined at 60 and 90 days intervals.

Propolis. We used ordinary propolis which we called heterogenous (H) thus pointing to its natural variable instable condition of unknown origin. Propolis H used in our experiments came from heterogenous mountainous flora. It was processed to obtain two pharmaceutical preparations : pills and aqueous solution. The pills called Propolis H_1 were prepared by physical purification of high quality propolis which was then treated by CO_3Na_2 at 30°C and the substance obtained was divided into pills of 1 g each. The solution called Propolis H_4 was obtained from high quality propolis which was purified, washed with an alcoholic solution at 30° and treated with distilled water (1:1).

Placebo pills were prepared from brown bread crumb well kneaded and divided into equal pills and placebo solution from clear water.

The symptomatic and etiopathogenic *chemiotherapy* consisted in associating drugs produced by U.M.B. of Romania and was used to stop crises, to remove the endocrine component which infected the spasmophilic ground and to prevent synthesis of prostaglandines, tissues hormones, and chemical mediators, which when in excess cause bronchial asthma.

Results

Group A who received buccal and nasal treatment with propolis associated with buccal chemiotherapy gave the best results : 100% (cure -4 cases, improvement-1). The results obtained with group B, which received a placebo buccal and nasal treatment associated with buccal chemiotherapy, were much reduced (30%), i.e. no cure — 2 cases ; 1 — improvement ; 3 cases — no results, which points out the value of propolis used to treat group A. Group C which was treated with buccal and nasal chemiotherapy alone gave also good results (60%) namely 2 cures, 1 improvement and no results in 2 cases.

The comparison of groups A, B, C shows that propolis was effective. It doubled the proportion of cures in group A by its therapeutical effects and not by psychological action. Buccal and nasal administration of propolis alone to group D gave poor results (20%) i.e. 1 improvement and no results in 4 cases. This actual but poor value is also pointed out by its comparison with group E treated with placebo and F which received no treatment, which recorded no positive results. After 95 days the positive results obtained with groups A, B, C, D, were maintained only in group A. This makes us assume that propolis increases the protective function and mechanism of the body and extends the duration of good results and this is possible thanks to the association of a naturist method with conventional chemiosynthetic ones.

Discussion

There are many works on the antiinflammatory effect of various categories of propolis (1, 2, 3, 5, 10, 11, 12, 15, 16, 17) particularly on affections of respiratory tract (1, 2, 9, 13, 17) and even on bronchial asthma in children (17) but none of them gives an estimation in comparison with synthetic medication and placebo. Similarly they do not mention the origin of propolis, nor the method of preparing and administering it, which is a natural complex instable product that varies with area, harvest, bee colony etc., and which we know only in part.

For this reason such works lose their scientific experimental value because they cannot be replicated and therefore they remain empirical observations. To provide a common basis for research we proposed (12) the following definition for propolis : it is a mixed heterogenous inequal instable complex of biological substances only partly known, which varies with harvesting and bee colonies, which they produce to protect the hive and themselves. Depending on the way it is prepared by bees — physiomechanically or chemiobiologically — propolis is a hard or slightly fluid assuming two main forms called by us superior and inferior depending upon the proportions of the same two main components : 1. protective juice of buds of trees in the area at the harvesting time ;

2. internal and external secretions of bees ; 3. pollen gathered by bees ; 4. beeswax secreted by bees. High quality (superior) propolis is that containing 70% of the first two components and low quality (inferior) propolis — that composed of the two latter components in proportion of 70%.

Similarly we suggest that conventional experimental methods be introduced provided that the matter, the material and the method of research are always specified and accompanied by results and discussions and double blind placebo is also used when possible. This work shows how apitherapy may be approached by modern scientific methods and only the low number of cases make the statistical significance be low. But we obtained some results that may be repeated and checked : 1. propolis H_1 administered *per os* and H_4 by nasal administration proved to be effective in treating bronchial asthma ; 2. a formula for preparing these two preparations ; 3. the confirmation of the action of propolis aqueous solution (1, 15, 17) ; 4. the effect of the treatment in 10 days or no effect (14). We think that propolisotherapy can be usefully applied to bronchial asthma. It has a pharmacodynamic, antiinflammatory, antiallergical, anaesthetic, locally eutrophic effect, a physiological hormonal immunological action which results in a slower, lesser global hypertonia of hypothalamohypophysis that increases the endogenous cortisone within the physiological limits and the protective mechanism of the body.

We also suggest that the poor action of propolis on this affection points to the need for associating it with the chemiosynthetic medication and at the same time for continuing investigations destined to identify, synthesize and concentrate its component active substances. Similarly efforts should be made to process, test and dose these components in terms of chemistry and pharmaceutics in order to obtain actual drugs useful to medicine.

This is the way all remedies wrung by man from the inexhaustible treasure of nature became medicines.

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APIPHYTOTHERAPY OF SOME INFLAMMATORY PROCESSES OF BUCCAL MUCOUS MEMBRANE

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The anatomic and functional conditions specific to buccal cavity explain the relatively great number of different types of affections of the mucous membrane of this cavity. Among them the inflammatory processes are prevalent.

As a rule the therapeutical methods applied in the inflammatory processes of buccal mucous membrane result in the cure of lesions. But the etiopathogenic diversity still requires a strictly enough individualization of the treatment depending upon clinical aspects and results of the laboratory tests. For instance an improper choosing of antibiotics for the treatment of an ulcero-necrotic stomatitis will increase the unbalance already existing in the microbial populations and thus aggravate the disease.

In the circumstances the possibility of having a more effective drug to be applied in a larger number of clinical forms is of particular interest and the apiphytotherapeutic preparations seem to have this advantage.

For the treatment of the affections of buccal mucous membrane we used 2 gallenic forms obtained by associating under complex technological conditions extracts of propolis and bee bread with certain extracts of plants.

For local application a flavoured brown coloured suspension which had a piquant taste was used to paint the buccal mucous membrane 1 to 6 times daily. The preparation has a strong antibacterial, antimycotic, antivirus, anaesthetic and vasotonic action being also a stimulant to the formation of epithelium.

In more serious cases we administered simultaneously the second preparation *per os*, 3 tablets daily, which we consider to have antiinfectious, antiallergical and cytotonic properties.

This treatment was established for the following types of affections of buccal mucous membrane :

— ulcero-necrotic stomatites — 18 patients ;
— buccal recurring aphthae — 9 patients ;
— labial cyclic recurring herpes — 7 patients.

Once the apiphytotherapeutic treatment have begun no other medication was applied.

During the treatment no adverse phenomena were recorded.

Results

The 18 patients with ulcero-necrotic stomatites of whom 7 were women and 11 men, were 18 to 50 years old. The affections had appeared 4 to 15 days before, and in some patients the previous treatments had given no results. As for the clinical aspect of disease 8 patients showed localized lesions, in 3 patients these were generalized and in 7 others they were associated with the alteration of general condition.

The microbiological test performed for 15 patients revealed a polymorphous flora more or less resistant to usual antibiotics.

The apiphytotherapeutic treatment was applied locally to 11 patients and in association with general administration of the preparations to the rest.

The treatment resulted in a rapid alleviation of pains, the fetidity disappeared and the lesions showed a clear tendency to cure. Remarkable results were obtained with the patients whose general state was very altered and who showed lesions caused by a microbial flora resistant to usual antibiotics.

We also point out the favourable effects of apiphytotherapeutic preparations on the buccal necrotic lesions in some patients with acute and chronic leukaemia. Among them the case of C.C., a woman 46 years old suffering from lymphocytic leukaemia for 4 years and sensitive to corticosteroids and chlorambucil seems to be particularly interesting. During the last year she showed generalized tumorous ganglionary masses, hepatosplenomegaly, hypertrophy of Waldeyer ring and a serious hypogammaglobulinaemia.

She had also petechiae disseminated on all the surface of body.

At the level of the lower lip to the right side she had a nodular tumorous formation of remittent consistency which disappeared without limits in the neighbouring tissues. Under the external third of lip there was an ulceration whose basis was harder and the edges were irregular covered with crusts. The surrounding teguments were congested. She did not show spontaneous pains on pressing the respective zone, but had a pruritus with perilesions. Numerous mobile painless ganglions of variable size could be touched in the bilateral submandibular and latero-cervical zones.

The endo-buccal examination revealed the hyperplasia of marginal parodontium which was congested with ulcerated zones at the peak of papilae particularly in the lower frontal region. The pharynx was also congested and the lymphatic formations in the Waldeyer ring showed a marked hyperplasia.

Haemogram : Hb — 8.3 g%, leucocytes — 300,000/ mm^3 with 99.5% tumorous lymphocytes and 0.5% granulocytes ; thrombocytes : 70,000/ mm^3 ; settling rate — 62/115 mm.

The cytological test of the smear obtained by scraping the surface of ulcerated zone revealed the presence of big tumorous cells with budded nuclei or plurinucleated cells among lymphoblasts and granulocytes.

Besides these giant monstrous tumorous cells one could also notice frequent neutrophile granulocytes in course of phagocytosis. In the cytoplasma of neutrophile granulocytes the ingested and destroyed elements were nuclei from tumorous cells and leucocytic lymphocytes that lost their normal function. The discovered image showed a pseudolupus phenomenon in which the ingested nucleus did not suffer changes in the homogenization of structure nor the loss of colouring as is the case with the lupus cell following the process of nucleolysis and depolymerization of DNA.

One can see the normal or incompletely homogenized structure of the nucleus of ingested lymphocyte, which we consider to be a protective reaction of the body consisting in the phagocytosis of diseased cells by the sound neutrophile polynuclear cells.

It should be noticed that the smear was taken after several days of local apiphytotherapeutic treatment.

The 9 patients with buccal recurring aphthae who were all men aged 30 to 64 years, showed lesions resistant to previous treatments with a very high frequency of recurrences (from 2 to 36). The various treatments applied to them did not remit the lesions.

The application of apiphytotherapeutic preparations by the method described above gave good results materialized in :

1. almost sudden disappearance of smartings after mere painting of lesions.

2. the reduction of the interval between the appearance of lesions and the formation of epithelium to 2—3 days in comparison with 10 to 16 days when non treated.

3. the diminution of frequency of recurrences in 2 cases and the cure in 7 cases.

In the other patients (5 men and 2 women) with labial cyclic recurring herpes the apiphytotherapeutic treatment gave similar results. The smartings and the pruritus disappeared in a few minutes, the complete cure took place in 2 to 3 days, the interval between recurrences increased and the intensity of subsequent pushes diminished, the acceleration of cure was also noticed in one case of intercostal herpes zoster.

Discussion and conclusions

The good results obtained by application of apiphytotherapeutic preparations to some types of inflammatory affections of buccal mucous membrane, that were different in their etiology and pathogenic mechanisms, cannot be explained but by taking into account the specific composition of preparations. These being obtained from natural products contain a large number of substances and biologically active groups which intensify mutually their pharmacodynamic action and chemical protection.

The analysis of the data supplied by clinical observations and laboratory tests make it possible to understand to some extent the main mechanisms of action of the two apiphytotherapeutic preparations.

1. Their antibacterial and antimycotic action is very strong in both *"in vivo"* and *"in vitro"* experiments with a large number of bacterial species. The preparations are also effective in controlling micro-organisms resistant to antibiotics ; they are more active in *"in vivo"* experiments probably by their activating some protective functions of the body.

2. A direct antivirus action is also possible in cases of recurring labial herpes and herpes zoster although we have not yet a confirmation by laboratory tests which will be the object of future studies.

3. The local anaesthetic contact action is obvious and strong enough, it being responsible for the alleviation of pains and pruritus. In this

way the vicious circle that cuts off the local neurovegetative distony is also interrupted.

4. The energetic action that favours the formation of epithelium is the main factor determining a rapid healing of ulcerous lesions.

5. The antiallergical action, which is easy to observe clinically seems to be to a lesser extent antihistaminic, because it is possible that a regulating capacity of the immunizing response should exist.

6. The cytotonic action shows itself conspicuous depending upon the tissues at the level of which it is exerted. We could mention for the time being the capacity of preparations of stimulating the haematogenic marrow and that of normalizing the activity of the main endocrine glands. One can also notice an increase in the general tonus of the body and an improvement in the output of metabolic cycles probably due to an enzymatic process.

7. The several types of action of the apiphytotherapeutic preparations develop simultaneously, wed to one another harmoniously and complete mutually.

Taking into account these data as well as the absence of adverse phenomena we recommend the apiphytotherapeutic preparations as a useful remedy to the inflammatory affections of buccal mucous membrane.

OBSERVATIONS ON THE TREATMENT OF GLOSSODYNIA WITH PROPOLIS

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Glossodynia is defined as a persistent painful sensation of tongue and buccal mucous membrane (BATAILLE, DESNOZAILLES, MUGNIER). It has been known for several centuries, the first reference dating from 1620, when VIGEN described it under the name of *Rhumatismus linguae*. During the 19th century there appeared numerous references which called it glossalgia (BRECHET, 1817), lingual neuralgia (VALLEIX-HALLIDAY, 1841). The dermatologist of Vienna, KAPOSI created in 1855 the present-day term, glossodynia, nearly unanimously accepted nowadays.

Considered for a long time a mere unexplained painful feeling, on closer examination one can see that this affection raises numerous problems as concerns its etiological and physiological mechanism and up to the present its therapy is not yet established.

Indeed, a closer examination shows besides the painful sensation, which is the main feature of clinical picture, an inflammatory process of the tongue, the hypertrophy of filiform papillae which are red and painful on feeling, a marked sublingual drawing, a global tumefaction of tongue with engraved dental stamps and sometimes an aspect of geographical or pilose tongue.

The objective and subjective modifications described above are caused by extremely varied factors such as : dental alveolate lesions, changes in the salivary pH, infections with *Candida albicans*, intolerance reactions to resins of dental prothesis, infections caused by a pharyngeal focus, changes in the balance of buccal bacterial flora as a result of the decrease in the salivary lysosime, gastro-intestinal disorders, avitaminosis B (lactoflavine), anaemia and neuro-psychical factors.

In the light of the foregoing glossodynia appears as a complex affection in which the objective changes bring about and later on maintain painful subjective phenomena.

Its numerous aspects interest at the same time the stomatologist, the dermatologist, the specialist in internal medicine and the psychiatrist.

The treatment of glossodynia is first etiological aiming at removing the causes favouring its appearance and then symptomatical aiming at reducing the local inflammatory phenomena and pains as a subjective symptom. In this sense there are used various therapeutic means : antibiotics, antimycotics, vitamins of group B, physiotherapy, corticosteroids, local anaesthetics, medicines acting on central nervous system, antineuroplegia medicines etc.

None of these therapeutic methods gave satisfactory results, glossodynia remaining one of the difficult problems of medical practice.

Given the limited therapeutic results obtained up to the present, we tried to include in the treatment of glossodynia new preparations that act specially on bacterial and mycotic infections and have also antiinflammatory and local anaesthetic effects.

To this end we chose propolis which, thanks to flavones, aromatic acids, enzymes and other active pharmacological substances it contains, has an antibacterial, antimycotic, antiinflammatory and local anaesthetic action and possesses to a great extent the qualities we are interested in.

Material and working method

Our study included 50 patients suffering from glossodynia, all of them adults aged 40 to 75 years. There were 46 women and 4 men whose disease had appeared at least 1 year before.

The clinical examination pointed to the presence of usual etiological factors : buccal infections with *Candida albicans* — 32 cases (64%),

decrease in or absence of salivary lysosome activity — 12 cases (24%), hypochlorhydria — 17 cases (34%), hypochromic anaemia — 2 cases (4%).

All patients showed a normal salivary pH and the bacteriological test revealed a normal saprophyte microbial flora.

A large number of patients (22) bore dental mobile acrylate protheses and other 18 patients fixed metal teeth.

All patients had been treated previously with different drugs: antibiotics, antifungal preparations, corticosteroids and antineuroplegia medicines without results.

Propolis was administered locally as mouth-wash under the form of preparations produced by the Apicultural Plant Complex of Bucharest: mouth water "Floral" and alcoholic solutions "Proderm 20%". 30 to 40 drops of solution were dissolved in 100 to 150 g of water. The treatment lasted 10 to 30 days.

To verify the results we took into consideration:

— changes in the subjective phenomena — disappearance of pain;

— disappearance of objective lesions, deposit and oedema;

— duration of cure.

The experiment was made by double blind. 20 patients received at the beginning a placebo.

Results

The results of the treatment with propolis can be statistically grouped as follows:

I. Cures: 16 cases (32%) with disappearance of subjective and objective phenomena for at least 3 months.

II. Improvements: 12 cases (24%) with visible reduction of subjective phenomena without changes in the local objective lesions.

III. No result: 22 cases (44%).

We also watched the effect of treatment with propolis on the buccal infection with *Candida albicans* but we could not obtain a sterilization of patients carrying such infection.

Conclusions

The local treatment of glossodynia with propolis was effective in 56% of the cases studied.

It is recommendable for its antiinfectious, antiinflammatory and local anaesthetic action.

The preparation is easy to administer with no risk or counter-indications.

The effectiveness of the treatment can be increased by associating it according to circumstances with other medications, with synergic, antibacterial, antimycotic and antiinflammatory action.

Although the number of cures and improvements was relatively moderate (56% of cases), the application of this treatment is indicated particularly in cases when the usual treatment has given no satisfactory results.

THE USE OF PROPOLIS-SPRAY FOR TREATING BURNS INDUCED EXPERIMENTALLY IN GUINEA PIGS

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During the last years propolisotherapy was put up-to date and reconsidered. The application of different preparations with propolis in the treatment of some cutaneous affections and lesions kept on being the object of attention. The data supplied by numerous works on the bacteriostatic, antifungal, anaesthetic, cicatrizing a.s.o. effects of propolis justified its use in the treatment of cutaneous burns. The results reported by M. I. ATTASOV et al. (1962—1970) who tried for 10 years the treatment of burns with propolis ointment are conclusive and promising.

That is why we decided to develop an easy and efficient method of applying propolis to burns. To this end we investigated its effectiveness by observing local symptoms and by making biopsies.

Material and method

The experiments were conducted on 20 guinea pigs of the same age and weight. The hair on the right side of animals was removed by using a depilatory ointment. After a superficial narcosis a burn was induced in the respective zone (5×5 cm) by means of an electrocautery, the duration of application being absolutely equal for all animals. The symptoms showed that it was a local intermediary burn (according to classification established by CHIRICUȚĂ et al., 1952). The treatment for the control group (10 animals) consisted of a daily application of an ointment with penicillin and sulphathiasol. The experimental group (10 animals) was treated by daily spraying with 10% propolis alcoholic solution pressure-bottled in metal bottles (spray). After 6 and 12 days respectively biopsy samples were taken from 2 animals of each group, which were analysed by the usual histological technique.

Results

Shortly after the burns have been induced the tegument in the respective zone assumed the aspect of a wet dirty coffee coloured bedsore with a congestive peripherical zone full of oedema and rare vesicles.

After 24 hours the burn in the group treated with propolis had a dry coagulated crust, and the peripherical congestive zone with oedema was much reduced. In the animals of the control group the wet necrosis, oedema and peripherical congestion remained unchanged.

After 6 days of treatment with propolis the burn was covered with a fine dry crust without symptoms of infection. Where the crust was detached one could see the specific aspects of the formation of epithelium with many fine hairs beginning to appear on the surface of burn. The healing took place "per primam" after 10 to 12 days of treatment,

the epithelium beginning to develop from both the peripherical zone of burn and the epithelial elements in the burned area, which had not been destroyed by cautery. The scar zones were reduced in size and showed no induration. The general condition of animals of this group was rather good, their behaviour and appetite being normal.

The animals of the control group, even after 6 to 8 days kept on showing a thick crust under which tissues were still inflamed and full of oedema. The spontaneous removal of the crust revealed a granular sore with numerous carnose buds and in most cases heavily infected. They were cured after 20 to 24 days by a slow cicatrizing thanks to the force of granular retraction and centripetal formation of epithelium. The scars were thick and indurated, and numerous zones had no hairs. It should be mentioned that the animals of this group showed an altered general condition. They were depressed and fed with difficulty and for this reason they lost weight.

The microscopic examination of biopsies taken after 6 days revealed in the control group a strong reaction consisting of morphological changes such as congestion, lymphohistocyte infiltration and a great deal of granulation tissue. In the animals treated with propolis these aspects were very discreet and the process of formation of epithelium was visible. The biopsies performed after 12 days revealed in the experimental group a visible healing process in progress whereas in the control animals the granulation tissues and the typic aspect of infection persisted.

Discussion

We think that certain properties of propolis are responsible for favourable results of its use in the treatment of burns, namely :

propolis has an antibiotic effect on numerous pathogenic germs (*Staphylococcus aureus, Staphylococcus viridans, Staphylococcus haemoliticus, Proteus vulgaris* etc.), sometimes surpassing the action of some antibiotics such as penicillin, tetracycline and ampicillin (I. S. ALEXANDROV, L. N. DANILOV, 1975) ;

propolis has also a visible antifungous action particularly on dermatophytes (V. F. BOLSHAKOVA, 1975) ;

it is also a local anaesthetic (T. TSAKOFF, 1975), a stimulant of the tissue macrophages (I. S. ALEXANDROV, L. N. DANILOV, 1975), the non specific immunoreaction (G. VELESCU, M. MARIN, 1975), the formation of the epithelium spores (J. SUTTA et al., 1975 ; N. APIETROAIEI, E. ILIESCU, 1975) ;

besides we must not ignore the content of microelements and vitamins of propolis because, as is known, vitaminotherapy (Vit. C, B_1, A) is particularly important for the treatment of burns.

As to the pharmaceutical form of use of propolis in the treatment of burns in animals we preferred the spraying, this being easy to apply by anyone. The fine film of propolis remaining after evaporation of alcohol has also a protecting effect and restrains exudation of plasma. We consider that propolis added to ointments has disadvantages because it does not favour the drain of wound, and besides it can even facilitate

its infection. The results of the investigations conducted by P. LAVIE (1975) show that the antibiotic principles of propolis are thermostable and are better preserved in alcoholic solutions than in other pharmaceutic forms.

Conclusions

Sprayings with alcoholic solution — propolis-spray — proved to be particularly effective in treating burns of an intermediate degree induced experimentally in guinea pigs.

We think that the antibiotic and anaesthetic action of propolis which stimulates the regeneration of epithelial and conjunctive tissue is responsible for these results.

We also think that the application of propolis in this way could be tried together with the other common therapeutical factors in the treatment of accidental burns in man

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THE USE OF PROPOLIS IN DERMATOLOGY

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In the dermato-venerics research Institute in Gorki, the products on propolis basis are used since long ago in the treatment of some dermatoses with various ethyo-pathogeny and clinical peculiarities (deep trichophysis, hyper-keratoses, skin tuberculosis, alopecia). For the treatment of deep trichophysis in the pilous area of the head (V. F. BOLSHAKOVA and I. V. VINOGRADOVA 1960), the 50% propolis ointment was used, being prepared with plant oil boiled at small fire or prepared with raw propolis solubilized in 96° alcohol partially evaporated until it became ointment.

110 pacients affected by deep trichophysis were treated, out of whom 92 had vesicles with focuses in the pilous area of the head and 18 had parasitory sicosis.

The ointment was applied on lesions, in thick layer under greased paper. In the first few days, an intensification of the swelling response was observed but it was attenuated in 3—5 days. The infiltrate was resorbed, the itching ceased, the pains calmed down or disappeared completely. The treatment had positive results in all pacients. In the majority of them, the fungi disappeared in 15 days. There were no alterings of the rough scars on the former lesions neither were any recurrance.

Taking into account the pronounced analgesic and anaesthetic effect of propolis, we used 1 : 1 propolis alcoholic solution as basis for preparing keratolitic ointment used for unsticking the epiderma in the case of hyperkeratoses and epidermophyses. (V. F. BOLSHAKOVA, A. M. IVANOVA, E. M. PEKKER, 1966). As keratolitic remedy, we used salicylic acid.

We treated 300 pacients having different forms of epidermophyses and hyperkeratoses of the soles. The ointment, applied on the hyperkeratosis focuses without any preliminary preparation of the area, in a thick layer, under plaster, was kept 3—5—7 days. I applied the 50% propolis salicylic ointment, depending on the clinical responses and on the character of the hyperkeratosis : first in 108 pacients, once : twice in 127 pacients and more times (3—4—6 times) in 65 pacients.

The 50% propolis salicylic ointment was superiour as compared to other keratolitic ointments because it gives no subjective sensations and it has profound destructive local effect.

The 50% propolis extract in ointment and the native propolis dissolved in alcohol included in seed butter, were also used in the treatment of the verruco-infiltrative forms of skin tuberculosis (V. F. BOLSHAKOVA, B. S. TIKHONOV, 1962). 50 patients with verrucose and infiltration clinical manifestations were subjected to the treatment.

The ointments were applied in thick layer under a plaster or under greased paper in verrucose forms, for 2—3 days.

In the case of the pacients with infiltrate they were applied daily. In some of the pacients, the 50% propolis salicylic ointment was first applied for the quicker distroying of hyperkeratotic prolipherations and only then, the treatment with soft propolis extract ointments was used.

They brought about the disappearance of the pus secretion and the total resorption of the infiltrate. The treatment lasted between 1 and 2 months. As a consequence, in 38 pacients the clinical curing began, in 6 of them a major improvement of the condition was noticed and in other 6 a slight improvement was found.

In the treatment of skin tuberculosis we do not consider the propolis as the main means of curing, however its analgesy in distroying the tuberculosis nodules, the healing of ulcerations and a quick cicatrization make it superior as adjuvant and is indicated for persons who cannot take synthesis antituberculous products for one reason or another.

For the treatment of total alopecia or in spots (V. F. BOLSHAKOVA and N. A. KUTOVA, 1964) the 30% propolis extract in ointment was used and also propolis alcoholic extract solution ; it was administer-

ed under the form of daily frictions on the pilous surface of the head, by vigorously rubbing down. Meanwhile, a rational feeding was prescribed, sports and no other medication.

Out of the 500 of patients, in 37% of the them disease lasted at most 1 year, in 40% — 2 years, in 15% — 6 years and in 12% — over 5 years. In some of the patients, the first signs of hair growth appeared after 2—3 weeks since the beginning of the treatment and in others after 1—2—3 and even 6 months.

Positive results of the treatment were found in 82% of the patients and negative ones in 18% of the patients.

One should have in view the possible peculiarities of composition and characteristics of different propolis samples depending on the place and period of harvesting. Different propolis varieties may have different allergic effects.

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TREATMENT OF SOME SKIN DISEASES WITH PROPOLIS

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Taking into account the antimicrobial, antipruritrous and epithelium growth features of propolis, as well as the results of the researches conducted by B. TIKHONOV and V. BALANIKOVA concerning the treatment with propolis of skin tuberculosis and by G. MUKHAMEDIAROV concerning chronic eczemas and neurodermitis, we studied propolis therapeutic effect in some skin diseases.

Between 1964—1972 I used propolis when treating 680 patients for different skin diseases, in the ambulatory clinics in Svetogorsk (see table). In 90.1% of the cases the result was positive. I used the propolis in ointment.

Disease	Nr. of patients treated	Recovery	Improvement	No effect
Eczema	170	90	65	15
Neurodermitis	312	152	146	14
Trophic ulcer	65	51	12	2
Other skin diseases	133	96	8	29
Total	680	389	231	60

For preparing the ointment (100 g), the propolis is purified; in an enameled bowl 80 g of vaseline heated on water bath at the temperature of 45°—50° are kept, and then 20 g of propolis are added. It is thoroughly mixed until one obtains a homogenous mass, it is then twice filtered through a gauze and then left to make sediments. The ointment should be kept in a cool and dark place, into a flask with ground-glass stopper.

For preparing the ointment, 100 g propolis are poured into a flask. 500 ml alcohol (96°) are added; the mixture is preserved in a dark place, being stirred now and then. It is filtered through a gauze after 10 days. The ointment is applied in a thin layer on the area of the lesion focus which is dressed. The dressing has to be changed daily.

The tincture (there is also a tincture with propolis) is taken daily 30—40 drops, 30 minutes before meals.

We treated patients with chronic eczema and neurodermitis with limited forms. Each process was localized especially on the back (dorsal) surface of the hand, leg, in the joint of the elbow (the poplitheal space). After 5—6 days of treatment an improvement of the condition of pruritus was found, the epidermis gets soft and more elastic.

After the treatment with the propolis tincture the patients observed they had a sound sleep and very good appetite.

Before having propolis, the patients with trophic ulceration of the shank had been treated with other methods, without efficiency. But due to the propolis ointment the wound is quickly cleaned, a granulation tissue appear and also epithelium on the surface of the wound.

THE CURATIVE FEATURE OF PROPOLIS IN DERMATOSES

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We studied some of the characteristics of the antibacterial effect of propolis for the pathogen staphylococci and its therapeutic effect in some skin diseases.

We found no natural resistance to propolis of the strains researched and during the adaptation of the pathogen staphylococci to propolis the resistance did not increase.

However, we found a strong antibacterial effect of propolis on the antibiotic resistant staphylococci strains. The bacteriostatic propolis doses ranged between 30—250—1000 microgrammes/ml and the bacterial ones 500—1000—2000 microgrammes/ml. The bacterial and bacteriostatic propolis concentrations reduce the biochemical activity of the pathogen staphylococci (they slow down the plasma coagulation, the manosis balance, the lactosis and sucrose balance), they also partially neutralize the toxins produced by staphylococci.

In the case of a staphylococcic septicaemia in albino mice, the phagocytary activity of the white cells increased after having applied propolis. In phagocytosis, first part is played by the macrophages. The propolis treated organisms were sooner pathogen-free than the control ones.

We determined the practical value of propolis products in different skin diseases. We had 112 patients, cut of whom 90 with profound pyodermites (furuncles, folliculites, hydroadenites), 12 of them with chronic furuncles of pyococcic ethiology, 10 with lupus.

In pyodermites and furuncles consecutive to shaving, we applied 20% propolis ointment, in lupus — a natural propolis product under a plaster. We found that propolis has also anaesthetic properties. The necrotic mass is easily cleared and the infiltrates are easily resorbed.

In all patients with pyodermites, the antibiotic resistant pathogenic strains of staphylococci were isolated from the attached tissues. The healing of chronic furuncles lasted 9—12 days.

In the case of lupus the attached areas became smooth and clean, the eruption disappeared and in 16—20 days, a fine scar could be seen.

The results obtained allow us to conclude that the essence of the propolis antibacterial effect is the reduction of the virulent character and of the staphylococcic fermentation, as well as the stimulation of the macroorganism phagocytary response.

USING PROPOLIS FOR LOCAL TREATMENT OF BURNS

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A great number of specialized works deal with the local treatment of burns, a matter which continues to be of present-day interest. In case of burns associated with necrosis and infection of wounds the method of treatment consists generally of the application of dressings.

Recently other methods of local treatment of burns have been tested consisting in the application of some antimicrobial preparations and other substances which destroy the early microflora and stimulate the regenerating process of tissues in the wound.

To this end A. A. VISHNIOVSKI and M. I. SCHEIBER (1975) recommend the use of an oleous balsamic emulsion for treating burns, prepared according to the formula established by A. A. VISHNIOVSKI (1937). Other vegetal balsamic substances whose main active factors are essential oils are used to treat the infected wounds. Such preparations are the fir-tree oil and ointment (A. S. TCHETCHIULIN, 1942), the essential and the eucalyptus decoction (M. A. ALIEV, 1950), the juniper oil (H. I. ERLICHMANN, 1944) and lately *Oleum hippophaeae* has been very much used.

M. F. KAMAEV (1970) uses a bacteriostatic haemopaste to treat burns and wounds.

Under the section of burns of the Institute of scientific research on traumatology and orthopedy of Gorki city the local treatment with propolis ointment (15%) prepared with vegetable oils (peach, apricot, sunflower oil) or lipids (M. V. KOLOKOLTSEV et al. 1965, N. I. ATIASOV et al. 1972) is widely used.

Propolis has a complex composition. It contains essential oils, balsamic substances, cinnamic acid, vitamins and other substances (R. E. KELLER, E. K. PRUDNITCHENKO, 1960). It possesses deodorant and anaesthetic properties and has bacteriostatic and bactericide effects on many gram-positive and negative micro-organisms (Z. H. KARIMOVA, 1960 ; V. P. KIVALKINA, 1960 ; F. T. KULEEV, 1960). The action of propolis intensifies the proliferation process of epithelium and the development of granulation.

One of the objects of the treatment of deep burns is to stop the infection of the wound not only in its degenerating and inflammatory stage but also in the regenerating one when all necrotic tissues are removed and the granulation begins to develop, because an abundant microflora in the wound prevents it from favourable development and if a skin transplant is necessary it becomes more difficult.

To intensify the antimicrobial action of the propolis ointment it was mixed with cetyl pyridine chloride at a concentration of 0.1%. Cetyl pyridine chloride is a preparation with a marked antiseptic effect belonging to the group of quaternary ammonium bases, which have a bactericide and bacteriostatic action on the gram-positive and negative micro-organisms (V. P. EREKAEV, M. P. GHERTCHUK, 1961, MÖLLER & RYDBERG, 1969, RYDBERG & AHREN, 1969).

The *in vitro* comparative study (12 experiments) of the action on the pathogenic microflora of 1) the ointment with 15% propolis without cetyl pyridine chloride (0.1%), 2) the ointment with 15% propolis mixed with furacilline at a concentration of 0.3% and 3) the ointment with 15% propolis without antiseptics, showed that the propolis ointment with cetyl pyridine chloride had the strongest antibacterial effect (table 1).

Table 1

DEVELOPMENT OF MICROFLORA AND THE ANTIBACTERIAL ACTION

Microorganism	Nr. of colonies of microbes under the action of ointment with 15% propolis		
	without antiseptics	with cetyl pyridine chloride, 0.1% concentration	with furaciline 0.3% concentr.
pathogenic staphylococcus	continuous development (over 1000 colonies)	$550+51$ $P<0.005$	$750+62$ $P<0.005$

We used the propolis ointment including cetyl pyridine chloride to treat more than 1000 patients aged 8 months to 87 years showing burns of the 2nd, 3rd A, 3 rd B degree with up to 75% of surface of body affected.

The patients with burns of the 2nd degree were treated by dressings with propolis applied after cleaning the wound to remove the content of vesicles and the remains of expoliated epidermis. In most patients we did not need to replace the dressing because the formation of epithelium began after 8 to 12 days.

The patients with burns of the 3rdA-3rdB-4th degree were treated with propolis ointment after detachment of the necrotic tissues thus stimulating the cure of the wounds caused by the burns of the 3rd A degree and preparing those with deep lesions for skin transplant. To this end, after a thorough cleaning of wounds we applied dressings consisting of 3 or 4 layers of gauze imbued with propolis ointment, which were replaced if need be (for instance when they were imbued with pus) at 1 to 2 days' interval. We used such dressings during the operation of free skin transplant for granular wounds.

The propolis ointment with cetyl pyridine chloride has a marked bactericide and bacteriostatic action and stimulates the regenerating process in the wound. Dressings with this ointment do not stick to the wound, stimulate granulation and are easy to replace because they do not cause pains, when removed, which is of paramount importance during the post operative period when the application of the dressing can displace the dermic transplant.

Our investigations showed that local application of the propolis ointment with cetyl pyridine chloride led to the improvement of the clinical evolution of wounds. At the same time the aspect of the granulations improved rapidly enough. They became pink or scarlet, regular in shape, moderately thick, but juicy, granulous and bleeded no more. The amount of secretion also diminished.

The cytologic study of wounds by the method of M. P. POKROVSKAIA and M. S. MAKAROV (1942) revealed a rapid favourable modification in the cytologic picture : the degenerating-necrotic and inflammatory types of the cytograms were replaced with the regeneration ones. The wounds showed a progressive diminution of microflora, insular forms of epithelium began to appear in the burns of the 3rd A degree and the peripheral zones of deeper wounds showed an epithelium in the making, which contributed to their better preparation for dermic transplant and for its complete fastening during the postoperative period.

The local treatment of burns with 15% propolis ointment with cetyl pyridine chloride in the concentration mentioned above gave no complications in the general condition of patients.

But the use of dressings with propolis ointment just before the dermic transplant proved to be detrimental because the thick layer of dressing applied on granulations prevented the transplant from adhering to the receptor bed.

That is why 2 to 3 days before operation and also on the day of transplant operation these dressings were replaced with others imbued with antiseptic solution (cetyl pyridine chloride solution 1 : 2000, furacilline, ryvanol, hypertonic natrium chloride solution etc.).

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TREATMENT WITH PROPOLIS AND OTHER HIVE PRODUCTS OF SOME OTORHINOLARYNGOLOGICAL AFFECTIONS

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Being convinced of the effectiveness of hive products in the treatment of some ORL affections and applying the experience of some authors of other countries we tried these products for 3 months under the Medical Centre of Apitherapy of the APIMONDIA International Beekeeping Technology and Economy Institute with the following results.

The ORL consulting room of the Institute had to overhaul during this period 128 patients 4 to 80 years old, namely 60 men and 68 women and the diagnoses are given in the table below.

No.	Diagnosis	No. of patients			Results			
		women	men	total	very good	good	poor	stationary
1.	Chronic allergical rhinitis	13	6	19	1	5	12	1
2.	Chronic hypotrophic rhino-pharyngitis	19	10	29	4	10	14	1
3.	Chronic simple atrophic rhinopharyngitis	8	7	15	1	7	7	—
4.	Ozena	1	—	1	—	—	1	—
5.	Chronic allergo-infected hyperplasic rhinosinusitis	5	6	11	1	4	5	1
6.	Chronic laryngitis (phonasthenia)	—	3	3	—	1	1	1
7.	Chronic medium suppurating otitis	5	4	9	4	2	3	—
8.	Chronic acute rhinopharyngotonsilitis	5	1	6	—	1	5	—
9.	Post traum-pharyngitis	—	1	1	1	—	—	—
10.	Cochleo-vest. syndrome	2	9	11	—	2	6	3
11.	Chronic hypertrophic rhinitis	—	6	6	—	1	5	—
12.	Physical debility	2	2	4	—	1	3	—
13.	External eczematous otitis	3	—	3	2	1	—	—
14.	Acute tonsilitis	4	—	4	1	3	—	—
15.	Operated and radiated ORL neoplasms	1	5	6	—	1	5	—

We will not dwell on the composition and therapeutic properties of bee products. They are dealt with at large in works by Romanian and foreign authors.

The diseases treated with hive products were as follows : trophic, inflammatory and common place infectious diseases and those resulting in a marked debilitation of organism.

We will describe the various affections and the way they were treated.

Rhino-sinus allergies. There were 19 patients who had suffered for many years from such affections and had been treated by all conventional anti-allergical therapeutical ways. We treated them for 3 to 4 weeks by instilling into their noses 2 to 3 times a day a solution containing propolis extract and vaseline oil. At the same time some of the patients were given for 10 days 3 times daily a ball of honey comb not larger than a plum to chew it for 15 minutes and then once daily for 10 days. All these patients received also a general treatment consisting of 10 drops of Proderm administered *per os* 3 times daily in a teaspoonful of honey with 2% royal jelly or 3 tablets of royal jelly or a vial of the same product *(per os)* daily. The treatment lasted 20 days. We chose for the treatment only the cases of pure allergy without any side infection. The results are as follows : a lasting cure in 1 case, a visible improvement in 5 cases, and a poor improvement in 12 cases. In 1 case the treatment gave no results.

Atrophies of the nasal and pharyngeal mucous membrane. This group included 3 categories of patients : chronic hypotrophic and simple

atrophic rhino-pharyngites and ozena. The hypotrophic affections were treated by instilling into the nose 4 to 5 drops of propolis solution 3 times daily, with nasal and pharyngeal paintings with 3% propolis alcoholic solution in glycerin after meals. To treat the simple atrophies we used besides the nasal instillations, paintings in the nose and pharynx with a 5% propolis alcoholic solution in honey containing 2% royal jelly applied also after meals. Ozena was treated with strong paintings with 0.5% propolis alcoholic solution containing vitamin A and streptomycin applied in the nasal fossae after removing the crusts and in the pharynx. All the patients of the 3 categories received also a general bracing treatment with pollen, honey and royal jelly and children received our typified preparations Melcalcin and Energin L. Five out of the 45 patients were cured for a long period, 17 showed important improvements, 21 poor improvements and 1 patient — no change.

When the local treatment was applied by the physician of the polyclinic the results were by far better than those of the treatment applied by the patient himself.

In 11 cases of *chronic allergo-infected hyperplasic rhino-sinusitis* we applied nasal instillations with propolis, nasal paintings with propolis solution mixed with honey and royal jelly and a general treatment with honey and royal jelly with the following results : 1 cure, 4 visible improvements, 5 poor improvements. In 1 case the treatment gave no results.

To 3 other patients with *chronic catarrhal laryngitis and phonasthenia* we applied a general treatment with pollen, honey and royal jelly with the following results : 1 cure, 1 visible improvement, and 1 poor improvement.

In 9 cases of otic and otomastoidean runnings after petromastoidean hollowing we used tents imbued with a 7% propolis alcoholic solution applied according to circumstances 1 to 3 times daily for 1 to 2 weeks. 4 patients were cured completely, in 2 suppuration ceased for a time and appeared again, so the treatment was resumed. In 3 patients the improvement was poor, that is the running was much reduced without disappearing.

In acute *inflammatory rhino-pharyngeal-tonsilitis thrusts* appeared in patients with chronic inflammation of upper breathing apparatus we prescribed gargles 3 times a day after meals, with 10% propolis alcoholic solution (Proderm) in doses of 10 drops in a cupful of common camomile. After gargle the liquid was swallowed. To some patients we applied the nasal and pharyngeal paintings used to treat the rhino-pharyngeal atrophies. The treatment applied to 1 patient who had been given antibiotics for 5 days without results made the affection yield in 24 hours. In 5 other cases the cure was complete after 3 to 4 days of local treatment.

One patient with *acute traumatic pharyngitis* — traumatism was caused by a foreign body — was cured completely in 48 hours by local treatment with paintings with propolis alcoholic solution in glycerin.

A group of 11 patients were treated for *cochleovestibular syndrome* with acouphene prevailing. Each patient received the general treatment

with our preparation Proderm (which is a propolis alcoholic solution) in concentrations of 10%, 20%, 30% and 50%. This was administered 3 times daily after meals in doses of 10 drops mixed with a teaspoonful of pollen and one of honey including 2% royal jelly. In 2 patients the acouphenes disappeared and the vertices decreased very much, 6 patients showed poor improvements and in 3 others the treatment gave no results.

In 6 cases of *chronic hypertrophic rhinitis* we used drops of propolis solution instilled into the nose and paintings with propolis solution containing glycerin or royal jelly with the following results : in 1 case an important reduction of the hypertrophy of scroll-bones and in 5 others a poor improvement.

Another group of 4 children (2 boys and 2 girls) under school age were suffering from bilateral latero-cervical polymicroadenopathy. They received the general bracing treatment with Energin L and Melcalcin with good results.

In 3 cases of *external diffuse eczematous otitis* we used with much success an ointment with propolis applied 1—2 times a day for 1 to 2 weeks.

One group of 4 patients with *acute tonsilitis* received a local treatment with gargles and paintings with propolis solution for 4 to 5 days. One patient was cured in 48 hours and the other in 1 week.

The last group of patients (6 in all) showed different *ORL neoplasms* operated and radiated with cobalt. Their general condition was rather poor. They were given 10 drops of Proderm 30, 3 times daily plus 3 teaspoonfuls of pollen and honey for 1 to 2 months. All of them regained their appetite, their sleep improved and they could feed better, so they gained in weight.

To some patients who showed an acute bronchitis we applied also a treatment with propolis syrup or propolis mixed with honey in doses of 3 teaspoonfuls daily (according to a very good formula of our chemist's). This had a good effect in a few days.

The table given below includes the age groups of treated patients.

From : 1 to 10 years	10 to 20 years	20 to 30 years	30 to 40 years	40 to 50 years	50 to 60 years	60 to 70 years	70 to 80 years	over 80 years
9	6	9	12	25	22	36	8	1

As it can be seen the patients of full and medium age are prevalent. It should also be noted that all the patients received a treatment with hive products only. We wished to see the accurate effect of such products and for this reason we purposely omitted to associate them with conventional medicines.

The short period of observations does not allow to draw final conclusions.

At any rate the increasing interest the public shows in the treatment with hive products and the generally good results obtained by us and other scientists abroad are promising and stimulate us to keep

on studying and extending this therapeutics under the medical units applying out-patient treatments.

But at the same time we should not neglect to test the sensitiveness of some persons to certain bee products particularly to pollen and propolis. Among the patients treated by us we had only 2 cases of allergy to propolis but with symptoms of minor importance, which yielded immediately after the treatment was interrupted.

We also noticed that a longer treatment with propolis caused in some patients a diminution of blood pressure. That is why we did not prescribe such a treatment for those with low blood pressure or we used it cautiously and checked their blood pressure periodically. It should be noted that patients who required an operation (ablation of nasal or auricular polypus etc.) received the apitherapeutical treatment only after they had been operated.

In conclusion we consider that the therapeutics with bee products of good quality applied under the control of the expert physician to well selected cases gives remarkable results. At the same time the patients should be enlightened as to the bee products that they are not an all-heal and they should be applied only to those the experience indicated to be able to support such a treatment.

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TREATMENT WITH PROPOLIS OF MONILIASIS AND INTERTRIGO IN INFANTS

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Moniliasis, caused by *Monilia albicans* is a frequent disease in infants, as well as in adults. It is entailed by disbacteriosis especially after administration of antibiotics with a wide range of effects. It mostly occurs in the bucal cavity, on the face, on the mucous membrane of the tongue and of the palate. Colonies of bacteria appear either on large or limited zones thoroughly stuck to the mucous membrane. Often, the affected people would not keep the food in the bucal cavity ; also watery salivation and often bleedings at the nose and mouth would occur. Infants are agitated, anxious and would cry often. Sucking is difficult, the infant patients have insomnia and hyperexcitation.

The disease progresses slowly, and is resistant to classical treatments : alkaline solutions, borax, tripaflavin, gentian violet, Nystatin, etc.

Therefore we tested propolis in treatment. The preparation included equal parts of 30% extract in 95% alcohol, water, and honey.

The patients were 40 infants, 7—15 days old, of both sexes, suffering of moniliasis of bucal cavity. The preparation was applied by dabbing the infected zones of the bucal cavity, three times a day, half an hour before sucking. Treatment lasted for 3—5 days. Already on the second day the affected zones of the mucous membrane reduced full healing being recorded on the 4th and 5th day. Infants calmed, their sleep became normal, their agitation and crying discontinued, and they put on weight. Treatment was well tolerated.

After the treatment was over, no recurrence was recorded for the next 3—4 months of observations. Also, no secondary phenomena occurred, the medicine being well tolerated by the patients.

Concomitantly, in the control group (35 infants) treatment lasted for 10—15 days, with classical medicines : borax, glycerine, gentianic violet, tripaflavin, and Nystatin ; frequent cases of recurrence were recorded. Treatment with propolis has the advantage that has ready effects and above all eliminates recurrence.

The excellent results obtained, the simple method of treatment, and absence of secondary phenomena justifies us to recommend the treatment with propolis as the most efficient method of curing moniliasis — an often occurring disease in infants.

We have also tested propolis in treatment of intertrigo in 1—3 months infants of both sexes. Intertrigo is as frequent in infants as moniliasis. Its symptoms are congestion of the skin in the zone of the buttocks and hips. The skin becomes red, erythema and secretions appear, followed by secondary infections with pustules. Infants are anxious, agitated, cry frequently and have a restless sleep.

Propolis was administered as 30% ointment. It was applied on the affected zones twice a day, for 2—6 days, with a substantial improvement of the general condition of the patients being recorded. In the affected zones skin cicatrized, infants calmed, agitation disappeared and their sleep became normal. All infant patients cured completely, and no secondary phenomena occurred.

We compared the results of the treatment with propolis and that with sulphonamidic ointments and tetracycline. The latter was applied in 30 infants and lasted for 10—15 days. It resulted that propolis ointment assured faster and more efficient healing. The treatment is simple and can be made both at the hospital and at home.

We recommend general use of the treatment with propolis in the therapy of moniliasis and intertrigo in infants.

LOCAL TREATMENT OF CHRONIC ULCERS WITH EXTRACT OF PROPOLIS

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The therapeutic properties of propolis have long been known by folk medicine. Later on they were studied more and more thoroughly without reaching final conclusions and nowadays this produce is the most

interesting subject in apitherapy but at the same time it has given rise to much controversy.

The specialized literature has already supplied numerous data on propolis and besides it was the subject-matter of several symposia and congresses. That is why we shall pass directly to present the results we obtained by local application of certain extracts of propolis in treating some diseases.

The extracts of propolis we used were obtained in two ways : 1) by macerating propolis in alcohol 70° in proportion of 20 to 50% ; 2) by macerating propolis in *Oleum helianthi*, a method which has not been mentioned yet in literature.

We first prepared an alcoholic extract of propolis by using alcohol 70° and propolis in concentrations of 20%, 30% and 50%. We allowed it to macerate for several days after which the product was filtered through gauze. The fluid extract thus obtained can be used as such or to prepare a soft extract by evaporation on a water bath.

The soft extract was then experimented by incorporating it with different ointment materials in concentration of 2%. This mixing resulted in a homogeneous unctuous preparation of high quality easy to preserve and wash.

The ointments prepared from soft extracts obtained from 20% or 30% fluid extracts gave the best results, irrespective of the material serving as basis of the ointment. The 50% extract of propolis had not an increased therapeutic action and for that reason it was discarded.

Besides ointments we used fluid extracts either obtained directly by maceration or by diluting the soft extract at concentration of 1 or 2% in a mixture of alcohol and glycerin or alcohol plus glycerin plus acetone, or alcohol with glycerin and ether.

Among them the fluid extracts including alcohol and glycerin as solvent were the best tolerated as shown in the chapter on clinical experiments.

Both ointments and fluid extracts applied to the affected zone bring about a feeling of smarting which disappears in 1 to 2 hours and sometimes eczemas.

To avoid this undesirable side reaction and taking into account that both resins and essential oils are soluble in oleum, we prepared the extracts by using *Oleum helianthi* in the same proportions of 20 to 30% crude propolis.

The mixture of oleum and propolis is kept at 60—70°C for 1 hour after which it is filtered while hot. After cooling the preparation becomes homogeneous having the consistence of an ointment easy to apply to skin and preservable for a long time.

The clinical experiments show that this ointment gives the best results without any side effects.

We applied these preparations to 34 patients of whom 32 with chronic ulcerations on the shank and 2 with chronic ulcerations caused by radiations, in one of them localized on *planta pedis* and the other vulvar. The group included 23 men and 11 women.

The 2 cases of radiodermatitis were cured by applying preparations with propolis, which resulted in the formation of epithelium immediately after budding. It is known that tissues subject to excessive radiation necrotize and bring about atonic ulcerative lesions very difficult to treat or sometimes lasting for years running or even for life resulting in extensive spinocellular epithelioma. In both cases there was applied a pomade with alcoholic extract and glycerized alcoholic solution, which cured the lesions.

In our opinion the treatment of the shank chronic ulcerations gave excellent results. This affection is caused mainly by alteration of vascular processes which first involve a single sector of circulation but later on they cover the other vascular sectors too. Its main feature is spontaneous appearance of atonic ulcerations at the level of shanks. They can also appear following minimum traumatism and tend to extend. Sometimes after reaching a certain extent it stabilizes and is cured spontaneously. Besides the determining factor, the ulceration develops on the background of a balance — a tolerance — established between the body and the microbial infection, sometimes of mycotic nature, at the level of affected zone.

As a rule such affections are improved or cured after 50 to 70 days of treatment because the budding of ulcerous zone is slow.

The local and general treatments of these affections rely on antimicrobial, vasculotrope, antiexudation, stimulating and regenerating medication, on physical rest and declivitous position of lower limbs.

In all cases of shank chronic ulcers the local treatment was associated with a general treatment with vitamins, A, B, C, E, P.

Before using the preparations extracted from propolis we applied local compresses with boric acid (3%) in order to change the pH of the ulcerated zone, to remove secretions and necrotic rests from ulcerations. In some cases we made cultures before and after application of the extracts of propolis, which showed that infection is sometimes improved by such preparations.

After deterging the ulcerations extract of propolis was applied on sterile gauze previously covered with a perforated cellophane sheet or directly on cellophane covered with gauze. In all cases the development of buds was good but eczemas were more frequent and exudation richer. By removing the cellophane the frequency of eczemas decreased as a result of the decrease in exudation.

The best results are obtained with the application of paintings for 48 to 72 hours, larger intervals bringing about eczemas which require temporary interruption of the treatment and application of compresses with 3% boric acid, 1% gentian violet, fenosept or metosept and possibly application of cortizonic ointments at the periphery of lesions. Once the eczemas have improved one can resume the treatment with propolis preparations.

In all cases dressings were changed at regular intervals of 48 to 78 hours.

The application of these preparations results in an intense and rapid budding, but the formation of epithelium is less intense. After budding

the development was slower and the process of formation of the epithelium required several procedures such as the chemical cauterization of the luxurient buds with silver nitrate or the application of red cell or fibrin powder or self graftings.

In testing these preparations with propolis the following indices were taken into account :

— therapeutic effectiveness ;

— absence of side subjective and objective effects.

We obtained very good therapeutic results with the alcohol and glycerin alcohol solutions, the ointments 20 to 30% irrespective of the constituent of ointment, and particularly with the oleic extract.

As to the side effects consisting in the appearance of smarting or pain feelings at the level of ulceration, pruritus or eczemas following the application of preparations, it should be noted that they are present in all cases but less marked in case of oleic extracts provided they are fresh. If the oil becomes musty it will result in the appearance of irritations, unpleasant sensations and possibly even eczemas.

The way propolis acts to give these therapeutic results is very difficult to explain. One can assume that its antimicrobial, antimycotic effect jugulates the infection process at the level of ulceration by interrupting one of the links of pathogenic chain. The removal of infection by discontinuing the links of pathogenic chain favours the regeneration of tissues, this being induced and increased subsequently by the active substances included in propolis because as is known the nucleus of active polyphenolic derivates linked to glycosides underlies rutin, a substance that has a flat capillarotrope effect. Thus the improvement in the circulation following the remaking of capillary membranes, the absence of infecting factor (which was removed) as well as the stimulating process of the antibody activity and the intensification of phagocytosis induced by propolis can stimulate the regeneration of granulation tissues by proliferating fibroblasts and increasing the natural reactivity of the body ; the antiflogistic effect reduces the cell lysis processes and the blood irrigation once reestablished improves the metabolic processes at the level of cells and local tissues.

Therefore the function of the body of remaking and repairing tissues is resumed and results in cicatrizing the lesions, a process quite contrary to the pathogenic one that favours the appearance of chronic ulcers.

The removal of the chronic infectious focus means an improvement in the protective and regenerating functions of the body, an improvement in the blood circulation marked by the appearance of newly formed vessels and all this favours the reorganization of the basic substance disordered and injured by the chronic infectious, inflammatory focus and the serious metabolic disturbances that took place at the level of ulceration.

The improvement in the blood circulation reduces the anoxia of tissues and allows a gradually resumption of the activity of enzymes, most of them being in the bloody flux, and the use of plastic substances of the body.

The remaking of vascular systems by newly formed capillaries takes place from the depth to the surface and particularly from the periphery of ulceration to its centre.

The complete reestablishment of dermic circulation involves also the process of formation of epithelium, but this develops more slowly probably because of the absence of buds to favour this formation, knowing that the epithelium is formed as a result of the activity of the remained sebaceous and sudoriferous glands or pilose folicles still remaining in the ulcerated area. It is from this level that the process of formation of the epithelium starts eccentrically through the basic cells and covers gradually all the ulcerated area. In case these factors are lacking particularly in deep or recurring ulcerations the epithelium is found only at the periphery of lesions starting from the basic layer of teguments surrounding the ulceration, hence concentrically without appearance of any points of epithelium on the ulcerated surface.

The histological tests consisting of biopsies of tissues taken from some patients confirmed the regenerative effects of propolis, its stimulating action of proliferation of fibroblasts as well as its inflammatory properties probably linked to the flavones it contains.

The results obtained with the 34 patients treated as described above were as follows :

— cure — 15 cases ;
— intense budding without eczemas or they were of minor importance — 13 cases ;
— less intense budding with eczemas — 6 cases.

THE USE OF PROPOLIS OINTMENT IN THE TREATMENT OF WOUNDS WITH GRANULATION : THE 10 YEARS EXPERIENCE IN THE RUSSIAN CENTRAL HOSPITAL FOR THE TREATMENT OF BURNS AND SCALDS

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One of the main problems of the postoperatory treatment in the patients with deep burns, successfully applied in the central Russian hospital for the treatment of burns in Gorki, (N. I. ATIASOV, 1962—1970) in the hastening of a complete cicatrization of wounds in the granulation stage, even of the very large ones (in the first 2 months after the trauma) in order to stop the development of irreversible alterings in the body.

One may gain time by reducing the intervals — of 5—7 days — between the repeated plastic surgery of the skin. The success of the treatment depends on the methods of the general treatment (blood transfusions etc.) as well as on rational medical preparation of the wounds by repeated treatment during the daily dressings.

From among the products we administered topically when preparing the wounds for dermoplasty after burning, the wounds being in

the granulation stage, the most frequently used was the propolis ointment (M. V. KOLOKOLTSEV, N. I. ATIASOV and others).

The ointment which contains 15% propolis may be prepared with any fat or oil (animal or vegetal). Most frequently, the propolis ointment used by us was prepared with peach, apricot and sun-flower oil. For preparing the propolis ointment with vegetal oil, it has to be heated until it boils, then the propolis is dried, ground and cleaned of impurities. It is then dipped in oil, carefully mixed and heated again until it boils; the supernatant substances are removed, the mixture is filtered through a gauze and then cooled down.

The propolis ointment was used between 1961—1970 for the treatment of 830 patients aged between 1 and 87 years old, with deep burns which had covered up to 75% from the surface of the body. After having removed the tissues with necrosis from the burnt surface, we applied dressings formed out of 1—2 gauze strata, stuffed with 15% propolis ointment, because our observations showed that the 5—10% propolis ointment is less efficient and the 20—30% propolis ointment has an initiative effect. It has anaesthetic, bactericidal and regenerative effect on wounds (I. F. KAZAKOV, A. P. KALININ, 1957, E. V. GLAGOLEVA, 1960, G. Z. MUKHAMEDIAROV, 1960, V. P. KIVALKINA, 1960, F. T. KULEEV, 1960, Z. G. CHAPYSHEV, 1960). According to the remarks of A. A. KIRSANOV (1965) the propolis intensifies the epithelium prolipheration and granulation growth and limits the surface of the scars. It also improves blood circulation and lymphatic one and reduces the permeability of the vessels from the burnt surface.

In 53 patients who were subjected to propolis ointment, the microflora composition was controlled (qualitatively and quantitatively) — according to Z. E. MATUSIS et. al. (1970). The dynamics of the cytologic aspect was also studied (according to M. P. POKROVSKAIA and M. S. MAKAROV, 1942). The number of the minor elements in vegetative condition on cm^2 of the wound surface, was reduced — after a treatment with ointment — from 1500—7000 to 425 ± 27. For the intensifying of the antimicrobial effect, we included antibiotics in the ointment and tests were preliminarily made to see microflora sensitivity to antibiotics or to antiseptics (furacillin).

The cytograms indicated that under the influence of the ointment it appeared an obvious tendency of decreasing the number of neutrophyls and of increasing the histiocytes, fact which proved the activation of the regenerative processes in the wounds brought about by burns. It also calms down the pains and the propolis ointment dressings do not stick to the wound and do not provoke trauma on the granulations which is very important in the postoperatory stage for the skin transplants.

VI. PREPARATIONS WITH PROPOLIS

PREPARATIONS WITH PROPOLIS

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The results obtained so far concerning the chemical composition of propolis, and especially the results of the investigation of the biological and pharmacological effects of propolis show that propolis has the following effects : bactericidal and bacteriostatic, antivirus, fungicidal and fungistatic, as antiflogistic, antiallergic, dermatoplastic biostimulator, and local anaesthetic.

This array of properties of propolis attests to the logical decision made by the specialists in the domain of medicine when they started to produce preparations with it and use them for medical treatments.

At present there are two countries — USSR and Romania — where medical preparations with propolis are produced.

We shall briefly describe them. In USSR 10 preparations with propolis are produced.

The first — *PROPOLAN* or *PROPOLANAS* is produced in the Lithuanian SSR. It is used in treatment of burns of the Ist, IInd, and of zones wherefrom skin for transplants has been removed. It is administered under the form of aerosols. After administration, on the zone from which skin had desquamated, a thin layer is instantly formed, which protects the burn and the wounds from the impure air thus preventing possible infection. The components of the preparation have antimicrobial effect, help clearing of the burns and wounds and faster healing. Propolan facilitates the epithelization and granulation of the skin which heals in 6—7 days, sooner than under usual conditions.

The preparation has also a pain smoothing effect, hence of a local anaesthetic.

The second preparation with propolis is *VAJVA*. It is also administered as aerosoles. It is used as mouth disinfectant, especially for removing the foul breath caused by decayed teeth, in some affections of the digestive tract, and to remove the odour due to consumption of onion

or garlic. It also proved efficient in removing the smell after smoking and after alcohol drinks. Besides the deodorizing effect, *VAJVA* also has excellent prophylactic impact in some affections of the salivary glands. Its effect is relatively fast and it removes or attenuates the foul breath for several hours. *VAJVA* is produced by the "Spindulis" enterprise of chemical products at Vilnjus (Vilna).

The third Soviet preparation with propolis is *META*, produced by the same enterprise. It is similar to *VAJVA* but is used for removing foul smell from rooms — dwellings and offices. A dose of this preparation refreshes the air and a pleasant odor remains for 30 minutes. Besides the deodorizing effect, an important quality of *META* is its capability of killing 30—50% of the pathogen micro-organisms — staphylococci, streptococci, agents of diphtheria and dysentery and of some pulmonary affections. A package of *META* can be used approximately 50 times for a room of 15 sq m. Freon is used as aerosol substance. *META* also contains other aromatic substances which intensify the deodorizing effect of propolis. Being easily applicable, and having a fast effect and a relatively low cost it is more and more widely used.

Other preparations with propolis are the propolis extracts with honey, usually of 1% and 5% concentrations. Experimental evidence exists that these concentrations of propolis cause no alteration but on the contrary honey therapeutical properties are intensified.

Another preparation is oleum propolis — 2 : 10 mixture of propolis and olive oil. This yellow-greenish preparation is mostly used in dermatology in the treatment of skin affections.

For the same purposes, a 20% propolis ointment is produced in USSR. It is a propolis extract in ethyl alcohol. Its colour is light brown and has a pleasant odour with specific aroma of propolis.

The 2—4% alcohol solution of propolis, chiefly promoted by the Institute of Stomatology of Kiev, is mostly used in stomatological therapy, surgery and orthopaedy with many good results having been obtained. Its therapeutical properties are also efficient in treatment of soft tissues of the bucal cavity, of aphtha and of other affections and scars in the bucal cavity.

Another preparation is aqua propolis or propolis water, obtained by suspension of propolis in cold water. It is a light brown, bitter solution, and is used in the treatment of the above mentioned affections of the bucal cavity.

The tenth preparation produced in USSR is the propolis emulsion. It is white. It is used especially in oto-rhino-laryngological affections. The package also includes a spatula by which the emulsion is spread.

In Romania, the preparation *FLORAL* is widely used. Floral is a mouth water including propolis alcohol extract and extracts of cortex cinnamoni, fructus cariophylli, mentholated oil and eucalyptol. It has a disinfecting, deodorizing, and local anaesthetic effect. It produces a pleasant cool sensation, removes the foul breath, stimulates salivation, and by its antimicrobial substances prevents fermentation and decomposition of food remnants. It also acts as a local anaesthetic — in people

with tooth affections. *FLORAL* is available as concentrate, and before use it must be diluted : 30—40 drops in 100 ml water. Because the alcohol propolis solution is insoluble in water, a suspension (milky solution of propolis in water) is instantly produced, which is used for rinsing the mouth.

The good results obtained with preparations with propolis have confirmed its real qualities as a medicine and its place in medicine.

At present, propolis is recommended for relatively few and non-representative affections and purposes as compared with the existing possibilities, because of insufficient medical research. Our task is to further seek for new possibilities of using propolis as a medicine.

The first steps in using preparations with propolis for medical purposes have been already taken in USSR and Romania. We should consider the production of such preparations too, not to lag behind other countries.

THE ROLE OF MALT-EXTRACT ASSOCIATED WITH HIVE PRODUCTS IN APITHERAPY

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Nobody can deny the role and importance of the empiric medicine as well as of the medicines it employs and which science studied and gave them the chemical formulae.

The old women cured formerly people round their houses by applying preparations developed by themselves and sometimes they were called for even by people of more remote areas.

The present penicillin whose chemical formula is no more a secret derives from the old mould the old women passed through a flame and applied to wounds. The moulds (scientific denomination *Penicillium notatum)* came from wheat or other plants.

Erysipelas which is known as an infectious contagious skin disease affecting also the subcutaneous tissue, caused by streptococcus was cured by folk medicine by smearing the cheek with honey, whose antibacterial principles healed the wound. The same old women prepared a pomade from poplar buds which they used to cure haemorrhoids. Besides such pomades were sold by the drugstores under the name of *unguentum gemmae popoli.*

It is known that poplar buds contain a certain quantity of tannic acid. Likewise, such poplar buds in olive oil were used to treat tuberculosis, arthritis, rheumatism.

We do not deny the value of synthetical drugs and their effectiveness, but nobody can deny that those created by nature are more effective and appropriate to human body.

The great botanist Linné said : "In comparison with other living beings, the human organism needs fruits and cereals that are an ideal food to man".

Cuvier, the author of the comparative anatomy, also said : "It seems that the natural food of man must be composed of fruits, roots, vegetables and honey".

FISCHER, professor, University of Yale, conducted an experiment on some sportsmen : Those fed on fruits and honey could hold their hands horizontally for 200 minutes. Those fed on meat only could do it but for 20 minutes.

The former could genuflex 1800 to 2000 times whereas the latter could do it only 500 times.

All these examples prove that the vegetable diet is superior to the others based on proteins.

I considered this preambul to be necessary to show that hive products are the result of the bees' work that visit flowers, plants, fruits, in order to collect nectar and pollen.

By using the principles of the extracts of plants I developed an apitherapeutic preparation, the *malt extract*.

This is an extract of barley associated with propolis, pollen and honey which I used to treat dyspepsia and catarrhal bronchitis.

The malt extract stimulates the breathing apparatus. It is known that nicotinic acid is to be found in both plant and animal cells. The nicotinic acid (or niacin) does exist in cereals in variable proportions. Among the cereals used as food for children, barley is the richest in this vitamin, but it exists in the malt extract too ; although to obtain such extract barley must be fried, the nicotinic acid (which is a vitamin) is not destroyed and its effectiveness remains unchanged.

The malt extract contains 10.8% niacin.

As is known the active function of the myocardium muscle needs a large amount of nicotinic acid necessary to both foetus and adult, whereas the organs with a continuous activity such as diaphragm and adrenal gland, need a larger amount of nicotinic vitamin. The human organism being low in nicotinic vitamin we administered malt syrup in the diet. Honey does not contain nitrogenous substances (proteins) and combined with malt + propolis, its effectiveness in treating T.B.C. increases and thanks to calcium propolis does not cause allergies.

Here is a formula already tested on patients :

Tribasic phosphoric calcium ;

Propolis ;

Malt extract ;

Honey.

It gave good results in tuberculosis, rachitis and scurvy.

An emolient gargle was also prepared from barley, honey and water. This can be used before applying aerosols with propolis.

Under laboratory conditions we added experimentally to this composition lecithin extracted from yolk by the alcohol-acetone method because the ethyl ether-alcohol method is difficult.

Lecithin was experimentally tested in the clinics of Italy by SORONO, in France by GILBERT and FOURNIER ; LANCESSEAUX tested it in tuberculosis, neurasthenia, rachitis, anaemia and cancer, with spectacular results. All the patients gained spectacularly in health. Their appetite increased and after a month of treatment they had gained 3 kg each.

It is known that lecithin increases the coefficient of nitrogenous oxidation to prevent an increase in the uric acid.

We added to the preparation malt, propolis, honey, chlorate magnesia in proportions such as to influence phagocytosis that increases very much by multiplication of polynuclears.

Besides, to prevent radiologic irritations and allergies caused by tars included in propolis, we added chlorate magnesia. This pomade is able to favour cicatrization of the wounds removing the prolonged runnings. Chlorate magnesia is extracted from sea water by industrial methods because a laboratory extraction is very difficult.

The composition of the malt extract is as folows :

Glucose — 30%
Dextrose — 25%
Nitrogenous substances — 8%
Ash — 3.5%
Water — 30%

The chlorate magnesia was also added to a bee venom pomade which is used to prevent allergies.

Conclusions

The treatment of some patients with preparations with hive products (pollen, propolis, honey) mixed with soft or dried malt extract gave very good results. Thanks to the nicotinic acid of malt the allergies of patients sensitive to pollen disappeared, so they could breathe normally.

RESEARCHES ON SOME PHARMACEUTICAL FORMS WITH PROPOLIS

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From the researches conducted so far they found that for the treatment of different diseases propolis is used in hydroalcoholic solutions without indicating a constant preparation technique. Neither do the excipients used for ointment disperse the rough propolis homogeneously.

The preparations indicated in literature do not represent pharmaceutical forms with a well determined content of active substances so that one could better follow its therapeutic effect. That is why, in this work we intend to establish the technique of the preparing methods and also the control and preserving of some pharmaceutical forms propolis-

tincture, fluid and soft extract injectable solutions and ointments. One should also establish its domain of using them as medicines by laboratory and chemical researches.

Thus we obtained the soft propolis extract which helped us both in preparing the injectable solution (not met in literature before) as well as in preparing homogenous ointments.

These pharmaceutical forms have a definite content of soft propolis extract and may be used in certain affections in human and veterinary medicine.

Material and Method

As raw material we used propolis which was obtained from the Băneasa Apicultural Complex Unit. This propolis is a malleable, perfumed, light brown substance and it melts at 80°C. It was preserved in glass pots of brown colour with a tight closure. For preparing the tinctures and the extracts, propolis was scraped and sieved through the sieve II and III.

Preparation of Tinctures with Different Alcoholic Concentrations

The raw material was formed from :

— propolis (II) 10 g

— 30°, 50°, 70°, 95° alcohol q.s. ad 100 g.

The propolis and the extractive liquid are introduced in brown colour pots, with tight closure and are kept at normal temperature for 10 days, by stirring them 3—4 times a day. The resulted alcoholic solution is filtered through filter paper and the residue is washed with the respective vehicle until the weight is completed. The tinctures are preserved in dark flasks at the temperature of 8—10°C, for 7 days and then filtered.

The bacterial researches show the positive activity of the tinctures prepared with 70° and 90° alcohol and to a less extent of those prepared with 30° and 50° alcohol.

THE RATES OF THE TINCTURES PREPARED WITH DIFFERENT ALCOHOLIC CONCENTRATIONS

Specification	Alcohol 70°	Alcohol 90°
Aspect	clear	clear
Colour	yellow-orange	yellow-orange
Flavour	characteristic, fragrant	characteristic, fragant
Taste	slight keen taste	slight anaesthetic senzation
pH	5.5	5.5
Turbidity figure	5.68%	5.48%
Density 20°	0.883	0.883
Evaporation residue	30 ml%	20 ml%

General Responses

1 ml of tincture diluted with 10 ml of water gives an opalescent homogenous solution without precipitate. The opalescent solution clari-

fies with addition of concentrated alcohol or with unionic tensioactive (tween 20 or 80).

5 ml of tincture are mixed with 10% Na OH (pH 9—10); HCl is added and the liquid is filtered. The flocculations on the filter are identified by dissolving them in alcohol and colouring them with ferric chloride (R).

Preparation of the Soft and Fluid Propolis Extract

The method was the following:

— propolis II 100 g
— 70° alcohol q.s.

The propolis (100 g) is ground (sieve II) and introduced into a cellulosic cartridge which is then passed through a high cylindrical diacolator (35 cm high and 3.6 cm in diameter).

The product is wet with extraction liquid (little by little) until it begins to pour through the inferiour tap which is open and above the mixture, another liquid layer may be found. The tap is closed and the percolation begins after 24 hours. During the whole period of the extraction, the product is covered with the solvent.

Initially, 80 parts of extractive solution are gathered separately. The subsequent percolations are concentrated under reduced pressure at a temperature under 50°C until removing the solvent. The residue is dissolved in the first fraction and is completed with 70° alcohol at the weight of 100 g. The fluid extract is kept in a cool place for 6 days, then it is filtered. It is a clear liquid, red-brown colour with a keen taste and may be mixed with 70° alcohol; pH 5.7; d = 0.95; turbidity 10 ml%; 21.7% residue.

The soft propolis extract is prepared in the same conditions as the fluid is without separating 80 parts. The percolation is performed up to the exhaustion of propolis of its active principles (1 g of propolis, 6—8 parts of 70° alcohol).

The extractive solution evaporates and concentrates at a reduced pressure and at a temperature under 50°C. The performance in soft extract is 39.5%.

The soft extract is brown-red and has the consistency of honey, insoluble in water, soluble in concentrated and diluted alcohol, in propylenglycol, polyethyleneglycol 540, isopropilic and bensylic alcohol (which solution is mixed with castor oil) as well as in tween 80. It is partially soluble in mineral and vegetal oil, glycerine and hog fat. It is also totally soluble in semisynthetic glycerides.

Preparation of Injectable Solutions with soft Propolis Extract in Different Solvents

A. *Dissolving of the Extract in Propyleneglycol*

Extractum propolis spiss	20 g
Propyleneglycolum q.s. ad	100 ml

The extract and the double quantity of propylene-glycol are weighed in a porcelain capsule. It is triturated until one obtains a solution which is passed through a 100 ml flask. Some of the anhydrous solvent is gradually poured on the extract from the capsule, until the whole product is passed on into the graded flask.

The brown-reddish solvent is kept to rest for 24 hours, when it deposits a weak white-yellowish precipitate. After decantation, the solution is put in ampoules of 2 ml, they are then closed and sterilized by tyndallization for 30 minutes at 70°, 3 times at 24 hours intervals.

B. *Dissolving of the Soft Extract in Polyethyleneglycol 400*

Spiss propolis extractum	20 g
Polyethyleneglycol 400 q.s. ad	100 ml

The soft propolis extract is dissolved at cold in the conditions of the technique 1. A gluey, homogeneous, brown-reddish solution was obtained and it might be preserved for one year without depositing. It is then put in 2 ml ampoules, then closed and sterilized by tyndallization.

C. *Dissolving of the Propolis Extract in Castor Oil by Means of Benzyl Benzoate (a) or of Benzylic Alcohol (b)*

a. propolis extractum	10 g
benzilium benzoicum	40 g
Oleum ricini q.s. ad	100 ml

The propolis extract is dissolved in benzyl-benzoate at the temperature of 35—40°C. The solution obtained is mixed with castor oil (sterilized at 140° — 2 hours and cooled).

The yellow-orange solution is kept to rest for 24 hours, then ampoulled in 2 ml ampoules.

The laboratory experiments show that for dissolving 1 g of extract, 3 g of benzyl-benzoate are necessary. Also, by using a 10% extract, the solution obtained is gluey and it can be bottled. The soluble extract benzyl-benzoate cannot be prepared with sun-flower oil, case in which an opalescent oil results which in turn separates the extract under the form of brown drops.

b. extractum propolis	10 g
alcoholum benzylicus	30 g
ol. ricini q.s. ad	100 ml

The dissolving of the extract in benzilic alcohol leads to a clear solution, brown-reddish with the pH 6.

If mixed with castor oil (sterilized) one obtains a yellow-orange solution which should be ampouled in 2 ml ampoules.

Preparation of Ointments with Soft Propolis Extract Dispersed in Different Excipients

The ointments prepared with rough propolis, unhomogeneously dispersed in different bases were prescribed in literature : vaseline, lanoline-vaseline, hog fat, butter etc.

For preparing some ointments we used as bases :

a — vaseline-lanoline
b — hog fat-wax-lanoline
c — unionic emulsifying ointment
d — stearic acid, tween, span
e — hydrophilic ointment with polyethyleneglycol
f — bentonite gel

a. The excipient currently used in chemists's vaseline with addition of 10% lanoline as emulsioning agent.

spiss propolis extract	10 g
adeps lanae	10 g
vaselinum ad	80 g

The soft extract disperses in 20 g basis of ointment (10 g lanoline and 10 g vaseline) previously melted on the water bath and semi-solidified. After the homogenization of the ointment, the rest of vaseline is added and is mixed.

The mustard colour ointment has a fragrant flavour when the extract is well dispersed.

b. In order to establish a difference between the soft propolis extract dispersed in classical basis (ointment-vaseline, lanoline and hog fat), an excipient with hog fat was also prepared.

extractum propolis spissum	10 g
adeps lanae anhydricus	10 g
cera flava	5 g
hog fat q.d. ad	100 g

The extract is dispersed into a melted mixture and also semi-solidified by lanoline, wax and equal quantities of hog fat. The ointment is homogenized and the rest of fat is added. If the soft extract is dispersed in simple hog fat, at cold, one obtains in the beginning a homogeneous product. As it is mixed, the extract begins to separate.

The hog fat with 5% wax gives a soft mixture. The lanoline (10%) in hog fat improves the quality of the ointment.

The addition of 5% wax permits a good preservation.

c. The soft propolis extract being solubilized in tween 80, we used the unionic emulsifying ointment as dispersing agent. It has in his composition the tensioactive agent in the concentration of 10%.

The excipient has the following formula.

alcoholum cetylicum	25 g
tween 80	10 g
paraffinum liquidum	20 g
valselinum	45 g

The cetylic alcohol, vaseline, tween and the paraffin oil are heated into a porcelain capsule at about 80°C. The fluidified mixture is passed into a heated mortar and mixed until cooling, thus the ointment being obtained.

In the above mentioned excipient, 10% propolis soft extract is dispersed and one obtains an ointment with mustard colour. The ointment forms with water an oil in water emulsion, white-yellowish.

d. Another excipient which permitted a good dispersing of the soft extract, is stearic acid, together with the agents-tween 60 and span 60 :

extractum propolis spissum	5 g
acidum stearicum	12.5 g
span 60	10 g
tween 60	10 g
aqua q.s. ad	100 g

The stearic acid, the span 60 and the tween 60 are melted on the water bath.

Water is added at the same temperature with that of the melting point of the mixture, and it is homogenized. The white ointment disperses homogeneously at cold the propolis extract.

e. The propolis extract was also incorporated into a washable ointment basis with polyethyleneglycols. The basis is soluble in water, it is not fat, it adhers well in epidermis and can be easily removed by washing with water.

polyaethylenglycolum 4000	40 g
polyaethylenglycolum 400	60 g

The components are heated in water bath at the 65°C temperature and after fluidifying they are mixed until cooling. In this hydrosoluble excipient the soft 10% extract was dispersed. The ointment is olive coloured, homogeneous, it can be diluted with water when a yellowish-milky emulsion results.

f. Dispersion of the extract in 5% bentonite gel

bentonitum	5 g
ac. boricum	0.50 g
nipaginum	0.05 g
aqua q.s. ad	100 g

In water, when boiling, one dissolves Nipagyne and boric acid bentonite is added in small quantities in warm distilled water (50°C). It is thus preserved for 24 hours for hydratation. It homogenizes and it is completed with water at 100 ml.

In the white-yellowish gel, the propolis extract is dispersed and a homogeneous greenish ointment results. The bentonite ointment and the propolis extract may be diluted with water in many proportions.

Clinical and Lab Researches

We searched the microbial and antimicotic effect of different preparations with propolis by the technique of antibiogramms and fungigramms — the diffusiometric method.

The results obtained are included in table 1. The values of the table represent the average radius (expressed in mm) of the inhibition zone.

Table 1

RESULTS OBTAINED WITH DIFFERENT PROPOLIS PREPARATIONS — THE DIFFUSIOMETRIC METHOD

Preparation	I	II	III control	IV	V control	VI	VII control	VIII	IX control	X*	XI control
Trichophyton schoenleini	7	6	0	10	0	12	12	5	0	3	2
Trichophyton quinckeanum	10	8	0	8	0	10	10	12	0	2	1
Microsporum audouini	10	12	0	12	0	15	15	2	0	2	2
Microsporum canis	12	10	0	10	0	10	10	5	0	10	6
Trichophyton rubrum	16	12	0	12	0	8	8	10	0	10	2
Trichophyton roseum	18	12	0	12	0	12	12	12	0	6	6
Trichophyton mentagrophytes	15	5	0	5	0	5	5	10	0	2	2
Trichophyton mentagrophytes interdigitalis	10	5	0	5	0	6	6	11	0	1	1
Candida albicans	3	1	0	5	0	10	8	10	2	1	0
Staphylococcus aureus	5	3	0	6	0	10	5	0	0	0	0

The propolis preparations and the control solutions are the following :

1. Propolis fluid extract prepared with 70° alcohol ;
2. Propolis tincture prepared with 70° alcohol ;
3. 70° alcohol (control) ;
4. Injectable solution of 20% propolis extract in polyethyleneglycol 400 ;
5. Solvent — polyethyleneglycol 400 (control) ;
6. 10% injectable propolis extract solution in benzylic alcohol (30 g) and castor oil 60 g ;
7. Solvent — 30 g benzylic alcohol and 60 g castor oil (control) ;
8. 20% propolis extract injectable solution in propyleneglycol ;
9. Solvent — propyleneglycol (control) ;
10. 20% propolis extract injectable solution, in 40 g benzyl benzoate and 40 g castor oil ;
11. Solvent — 40 g benzyl benzoate and 40 castor oil (control).

From the analysis of these data, it results that the vehicles 3, 5, 9 have no antifungic effect. Vehicle 11 has a slight effect and vehicle 7 a good antifungic effect.

The preparations 1, 4, 6, 2, 8, 10, 11 had a chronologically diminishing antifungic effect.

The propolis extract applied locally or administered parenterally may give good results in the treatment of dermatomicoses.

The biologic experimentation of the injectable solutions proves that they are well tolerated by the animals from laboratory.

The propolis fluid extract and the propolis tincture in 70% alcohol have in vitro a remarkable effect against : *Microsporum ferrugineum, Trichophyton equinum, T. verrucosum, T. tonsurans, T. violaceum* (the technique of successive dilutions in solid medium was used) and *Epidermophyton flocosum (inguinalis).*

The preparations have a weak candidosic effect and do not inhibit the developing of *Penicillium notatum* and *Aspergillus.*

Notable variations in the antifungic effect of different propolis varieties were observed.

These preparations have also a weak effect against *Staphylococcus aureus.*

The propolis ointments were used in 10 and 20% concentrations, the former ones being better tolerated by teguments. There were no differences as therapeutic effect depending on concentration and in the basis in which the soft propolis extract had been dispersed.

In eczemas dermites one may see in some of the cases an exacerbation of the cutaneous process from the first applying and in others an initial improvement followed by exacerbation after 2—3 applyings.

In chronic dry eczemas one can see a considerable sedation of the pruritus, an improvement of the pathologic process, but no healings.

Appreciable results were also obtained in localized pruritus cases and nevrodermites where after 1—2 applyings, the sedation of the pruritus was obtained and after 4—5 days, an improvement of the cutaneous process.

The fluid propolis extract was used successfully in 50 cases of chronic leg and mouth disease under the form of daily paintings.

Conclusions

Out of propolis, from the hive, tinctures, fluid and soft extract of propolis were prepared. The soft extract was used for preparing some pharmaceutical forms : injectable solutions and ointments.

The best vehicle for preparing the tinctures, the soft and fluid extract was 70° alcohol.

4 injectable products with nonwatery solvents as vehicles, were prepared : propyleneglycol (a), polyethyleneglycol 400 (b) castor oil, by means of benzol benzoate (c) or benzylic alcohol (d).

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PHARMACEUTICAL PREPARATIONS WITH PROPOLIS EXTRACT USED IN THE TREATMENT OF CHRONIC PERIPHERIC PARODONTOPATHIES

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Chronic peripheric parodontopathies are widely spread affections and constitute a particular concern to stomatologists who must discover them in due time, determine the accurate stage of their evolution and diagnosis and establish the proper treatment.

A particular factor which creates inadequate conditions for the effective treatment of such affections is the fact that their beginning is more often than not insidious and their evolution is not visible, or their symptoms are rather poor, without manifest forms for several months or even years.

That is why although people take frequently medical advice chronic peripheric parodontites are discovered in a stage whose clinical picture is doubled by an objective advanced condition materialized in deep parodontal destructions difficult to master by conventional therapeutic means.

The successful treatment of chronic peripheric parodontites depends on the use of a number of therapeutic means as large as possible and on the discovery of lesions in their incipient stage.

Accordingly we decided to study the antiinflammatory effect of propolis extract in the incipient forms of parodontites : chronic peripheral skin-deep gingivitis and parodontitis.

In our investigations we took into account two factors, namely (1) the need for treating parodontitis in its incipient stage when the treatment is very likely to succeed and (2) finding and developing a drug particularly effective in treating the skin-deep inflammation of parodontism, making it yield completely to the treatment.

This paper gives the results of the study by clinical and laboratory tests of 30 patients suffering from chronic peripheric skin-deep parodontitis and gingivitis, who were treated with propolis ointment in concentration of 20% with alcohol 70% being used as carrier.

The treatment consisted of local application of ointment by painting the interdental papillae and the free position of gums and the inside of gum groove.

To determine as accurately as possible the antiinflammatory and antimicrobial effects of propolis extract the patients were subject to a bacteriological and clinical examination both before and after the treatment in order to compare the results.

The microbiological test consisted in studying directly under microscope the microbial flora in the inflammatory exudate taken before and after the treatment from the gum groove, and the cytologic aspect. The data of the clinical examination were compared to those of the bacteriological test. The *in vitro* antimicrobial action of propolis extract was also determined.

The clinical examination consisted in appreciating the stage of the peripheric diseased parodontium by P.M.A. index and the changes occurring in it following the treatment.

To comparatively determine the therapeutic effects of the propolis extract depending upon the time when they become visible we studied also the antiinflammatory effects of some organic compounds of vegetal origin and of zinc chloride, a substance of conventional use in the therapy of the inflammation of peripheric parodontium.

In this sense we give below in table 1 the necessary number of sittings in which propolis and vegetal extracts and zinc chloride were applied to make inflammation of gums yield. As it can be seen in the table the propolis extract works more rapidly, which results in a lower number of sittings and a larger number of patients being treated, namely 19 patients in 3 sittings as compared to 11 patients in 4 sittings respectively. The treatment with vegetal extracts and zinc chloride requires a larger number of sittings.

Table 1

Number of sittings	Number of patients		
	Treatment applied		
	Propolis extract	Vegetal extracts	Zinc chloride
3	19	20	8
4	11	29	13

The variation of the P.M.A. index in comparison with the use of propolis extract in the same patients was also studied.

We considered that the decrease in the P.M.A. index — which conveys the inflammation degree of gums — from 0 to 25%, shows a non satisfactory therapeutic effect and is tantamount to a *stationary* cli-

nical state. The decrease in the P.M.A. index from 25 to 75% shows a good therapeutic effect corresponding to a clinical state of *improvement* and if the index decreases from 75 to 100% it means a very good therapeutic effect corresponding to the clinical state of being *cured.*

The clinical study after treatment of the antiinflammatory effect of propolis extract in comparison with vegetal extracts and zinc chloride is illustrated by table 2.

As it can be seen in table 2 the most effective is the propolis extract with which we obtained 23 cures and 7 improvements or 76% cures and 14% improvements. When we used vegetal extracts we obtained only 53% cures, 41% improvements and 6% stationary cases.

As it can be seen in this table the application of the propolis extract resulted in the largest number of improvements and cures in comparison with the other tested substances, namely 77% cures and 23% improvements.

To illustrate these results we present the following case: a patient aged 37 with skin-deep chronic peripheric parodontitis. He showed a

Table 2

Number of patients			Clinical state after treatment
Treatment applied			
Propolis extracts	Vegetal extracts	Zinc chloride	
0	3 (6.1%)	6 (28.5%)	stationary
7 (23.4%)	20 (40.8%)	8 (38%)	improvement
23 (76.6%)	26 (53%)	7 (33%)	cured

generalized gum inflammation with tumefaction of interdental papillae and conjunctive tissue and presence of serifibrinous exudate. P.M.A. index before treatment : 56.

The cytobacteriological test revealed the presence of cocci in a great number, fuso-spiral associations and remains of epithelial cells.

Even the first sitting of treatment with propolis extract resulted in a visible improvement and this developed favourably until total disappearance of the signs of inflammation, the complete cure of the patient taking place in 3 sittings.

One could notice the normal aspect of interdental papillae and free edge of gums. The PMA decreased to 0, hence by 100% and the cytobacteriological test revealed a total disappearance of spiral fusiform association.

Another case showed visible signs of inflammation : congestion, papillary tumefaction, serofibrinous exudate, which points to a skin-deep chronic peripheric parodontitis. The PMA index before the treatment — 67.

The cytobacteriological test revealed frequent fusiform bacilli, an abundent fusospiral association, very frequent heaps of cocci, disintegrated red cells, remains of leucocytes.

After treatment, which consisted of the application of propolis extract in 3 sittings, the patient was completely cured. The PMA index decreased to 0, hence by 100%, and the cytobacteriological test revealed the absence of fusospiral association.

The results bring out the important antiinflammatory and antimicrobial properties of propolis extract, which being prepared from a natural produce and which does not result in creating antigens, can become, thanks to its particular therapeutical action, a useful tool for the treatment of skin-deep inflammatory affections of the peripheric parodontium.

CONTRIBUTIONS TO PREPARING ANTISEPTIC PILLS WITH PROPOLIS EXTRACT

(Abstract)

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The experiments aimed at obtaining pills with propolis extract which should ensure an antiseptic effect for a longer time in the mouth cavity, and in the pharinx. Thus we had to determine some specific parameters in pills for sucking as concerns the disaggregating time which has to be longer in this case (according to "Farmacopeea Română", IXth ed., the disaggregating time for pills is 30—60 minutes), the taste and a homogenous distribution of the soft propolis extract — a hydrophobic product — in hydrophilic solid excipients.

After many experiments, the technology of preparation of propolis extract pills was established on the basis of wet pelleting (granulating).

They have an average weight of 1.00 g, a homogenous aspect, 55 minutes disaggregating time, 12 kgf/cm^2 mechanic resistance (Pfizer device), 37% friability (Erweka device). The pills have good fragrance and they are easy to administer.

The antiseptic effect in the mouth cavity was already tested and clinical otorhinolaryngology tests are in further study.

AGAIN ABOUT PROPOLIS

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After having published the report "Propolis in Skin Diseases" (*Pchelovodstvo* 1971, nr. 6, p. 30) we received many letters in which we were asked to answer whether the propolis ointment might be ap-

plied in one disease or another and which is the way to prepare it. Some letters described some diseases and asked whether their treatment was adequate and should these patients use propolis etc.

Because I could not answer these letters I wish to remind people that without seeing and examining the pacient the physician cannot establish a diagnosis, he cannot see the evolution of the disease neither can he prescribe an efficient treatment. Despite the remarkable features of propolis it should not be considered a universal panacea.

The therapeutic features of propolis are well known since long ago. They are explained by its content of antibiotics, carbohydrates, vitamins, minor elements, minerals, resins, balms and many other substances. That is why the propolis was applied since very old times for treating wounds, ulcers, burns, chronic eczemas, lupus etc.

In the case one does not follow the physician's recommendations or applies propolis incorrectly, the disease might aggravate.

Here are some of the receipts for preparing propolis (ointment). The 10, 15 or 20% propolis ointment is prepared as follows :

1. In an enamelled bowl, 100 g vaseline or animal fat are melted. It is heated up to the boiling point, then the content is cooled up to 50—60°. To the cooled vaseline, one adds 10—15—20 g of propolis (depending on the necessary concentration) by grinding it and removing the visible waxes and mechanical impurities.

The mixture is again heated up to 80°, continually mixing it for 8—10 minutes into a covered bowl. The hot mixture obtained is filtered through a gauze filter and again mixed until it cools down ; when cooled, the ointment may be used for medical purposes.

2. The ground propolis without visible mechanical impurities or wax, is dissolved by boiling it in 96° alcohol in the ratio : 1 kg of propolis to at most 300 cm^3 of alcohol. A dense mass is obtained, easy to stretch, with a dark brown colour and a pleasant flavour.

As basis for preparing it one uses vaseline or vaseline + lanoline in the ratios 9 : 1 or 8 : 2. For 100 g basis, 15—20 g of prepared propolis are taken. The basis is melted on boiling water, one adds the preparation and one should stirr it now and again for 5 minutes until completely dissolved.

The bowl should be closed tight so that the chemical compounds should not volatilize.

After 10—15 minutes since the beginning of the cooling, the ointment is filtered through a gauze and put into a glass jar.

It may be preserved in dark, dry and cool places.

THE TECHNOLOGY OF OBTAINING SOFT PROPOLIS EXTRACT FOR PHARMACEUTICAL USE

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Investigations conducted in numerous countries brought out the therapeutical value of propolis and preparations with this hive product, and determined the conditions for their use in apitherapy. Their results are largely reported in numerous works. Propolis is widely used for apitherapeutical purposes under different forms such as granules, powder, soft and dry extracts as well as other numerous preparations. Under the Medical Centre of Apitherapy in Bucharest there were experimented several apitherapeutical preparations with propolis, namely : honey with propolis, suppositories, syrups, ORL preparations, ointments etc.

All these preparations were obtained by using soft propolis extract, which shows that this form is the most frequently used with best results. The soft propolis extract is a preparation obtained by selective extraction of active principles of propolis with alcohol being used as solvent. The solution is concentrated until obtaining a viscous mass containing 20% solvent at the most. Because the soft propolis extract is more and more used in manufacturing a great number of apitherapeutic preparations it was necessary to pass from the laboratory to the semiindustrial production which requires adequate equipment. The investigations conducted by the Institute of apicultural research led to the development of a technological method of preparing soft propolis extract including the following operations.

Selecting propolis

Propolis is a coffee-coloured brown solid heterogenous mass with greenish shades ; it has a marmorean aspect, a hard consistence, and includes many foreign bodies.

As to its physical and chemical composition it includes 50 to 55% resins and balsams, about 20 to 25% beeswax, about 15% essential oils and 5% pollen. The cakes of propolis differ in form and size.

To obtain a preparation as rich as possible in active substances it is necessary to select the cakes of propolis so that it should include the lowest percentage of beeswax, foreign bodies and other materials which could impair its qualities.

To determine this percentage propolis is first tested by organoleptic methods and when in doubt laboratory determinations are made by using samples taken from all parts of the cake.

The laboratory analyses aim at determining the percentage of propolis soluble in a solvent, that of beeswax and foreign bodies.

Grinding propolis

To obtain as high a percentage of active substances as possible and as rapidly as possible the cakes of propolis must be ground until obtaining granules of 2 to 4 mm, which offer a large surface of contact with the solvent thus facilitating extraction.

The grinding of propolis is a very difficult operation because it has a hard sticky consistency and for this reason the grinding machines in common use do not give satisfactory results with propolis.

Our experiments gave good results, the grinding of propolis being performed in two stages :

the 1st stage consists of cutting the cakes of propolis until granules become 20 to 30 mm in size ;

during the 2nd stage the granules are ground until they reach 3 to 4 mm in size. To perform the 1st operation we used a mechanical press of 150 kg/sq cm fitted with very hard steel knives. The cakes were pressed and cut in granules concomitantly. For the 2nd stage we used a machine provided with a hard movable drummer fitted with knives which during the turning of drummer pass through the knives of a fixed plate. By adjusting the distance between the knives, we obtained the desired grinding.

Maceration

The active principles of propolis were extracted by alcohol 90% in charges of 70 l of alcohol to 30 kg of propolis granules. The mixture was put into a stainless jacketed wall extracting apparatus heated by hot water at 40°C. The mass is agitated mechanically by a mixer, the r.p.m. being rather low (1 rpm.). Moderate agitation and heating of the mixture help improve the extraction. After 48 hours the amount of fluid propolis extract was about 75 to 80 Kg.

Filtering

To separate the non-dissolved residues from the propolis extract it was allowed to settle, then it was strained through a coarse vacuum filter and afterwards through a fine filter (filter paper) by a special apparatus provided with a vacuum pump.

Concentration

The amount of solvent in the soft propolis extract was reduced up to 20% by using a vacuum concentration apparatus. This consists of a stainless jacketed distillating still with hot water at 70 to 80°C. The moderate vacuum evaporation and heating help maintain the active principles of propolis and obtain a rapid concentration. Solvent vapours pass from the distillating still to a condenser consisting of a coil sunk into a container in which cool water is continuously running. The condensed material is collected in a stainless container provided with a vacuum pump.

From the initial amount subject to concentration we obtained about 26 kg of soft propolis extract. This was viscous, brown-reddish coloured, with a characteristic odour of propolis, insoluble in water but soluble in alcohol.

The quality of the product is checked by determining its density which must vary between 1.096 and 1.159, the amount and nature of flavones and the flavoured acids it contains.

Until its use the preparation is kept at room temperature packed in tight dark coloured glass or enameled containers.

The equipment for preparing propolis extract can be designed to meet the needs of any production, the flow remaining unchanged.

The soft propolis extract manufactured by us was used to produce a number of apitherapeutical preparations that were tested by the Medical Centre of Apitherapy in Bucharest.

Among them we mention

Syrup with propolis : it contains some of the components of propolis such as essential oils, balsams and particularly flavonoids and ferrulic acid, which give the preparation antivirus and antibacterial properties. It is used in certain affections of breathing apparatus with good results. It is a topic bactericide that regenerates the epithelium. Another preparation, "*Honey with propolis (2% and 5%)*" contains the same elements.

Tablets with propolis : they contain 5% propolis, sucrose and ingredients specific to tablets; they are used in the affections of bucal cavity and pharyngitis as an antiseptic and to relieve congestion.

Propoheliant : this is an oleous propolis solution which relieves congestion of the rhinal-laryngeal-pharyngeal mucous membrane and is used in acute and chronic rhinitis as an analgesic relieving congestion and regenerating the mucous membrane.

Mipropol : is a preparation under the form of suppositories and ovules containing propolis, royal jelly, pollen and honey. Thanks to its complex composition the preparation is widely used in inflammatory, erosive and disfunctional affections (prostate adenoma, erosion of cervix uteri etc.).

Apifort : is an ointment having antiseptic and cicatrizing qualities, and contains soft propolis extract, royal jelly and pollen extract included in an excipient easy to absorb. It gave good results in treating different wounds and particularly in those of face.

As a matter of fact the soft propolis extract is used to manufacture a wide range of drugs and beauty preparations, which cannot be described in this paper.

PROPOLIS ALCOHOL SOLUTION (PROPOLIS SPRAY) FOR PROTECTING BED-KEPT PATIENTS FROM CUTANEOUS INFECTIONS AND BEDSORES

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The well known antibacterial properties of propolis suggested investigations of its effects on preventing infections.

The tested preparation was an alcoholic propolis solution in concentrations of 5, 10 and 20% as a spray. The protecting effect of propolis was tested on 30 patients — 21 women and 9 men — aged 48 to 92 years. The patients were bed-ridden either because of plastered thighbone cervix fracture or neurological affections (hemiplegia, paraplegia) accompanied by psychic and serious cardiovascular troubles.

The protecting treatment consisted in pulverizing propolis spray twice a day on the dorsal regions in contact with the bed.

The treatment lasted 10 to 75 days. Only 4 patients showed bedsores during the treatment. They were depressed, showing a sphincter incontinence, serious neuropsychic and trophic troubles. The other 26 patients were perfectly protected. Even those showing a sphincter incontinence showed neither bedsores nor cutaneous infections during the protecting treatment. In 3 patients, after a few days from cessation of sprayings, numerous eschars appeared on their dorsal region.

In order to establish the protection mechanism of the propolis solution we continued researches in two ways :

1. The antibacterial effect of the solution *in vivo.*
2. The antibacterial effect of propolis *in vitro.*

The *in vivo* effect was checked by taking bacteriological samples from the sprayed regions after one hour, after 6 hours and after 24 hours after spraying and by counting the appeared microbial colonies.

These samples were compared with those taken before spraying, in the same patients.

We found that the samples taken 1 hour after spraying had a very small number of microbial colonies.

Those taken after 6 hours had a greater number of colonies and after 24 hours, the cultures taken from patients before and after spraying were identical.

The effect of the *in vitro* solution was checked by making the antibiogram of the samples taken from patients tegument before and after spraying with propolis solution, aqueous propolis extract and powder propolis.

The antibacterial power of these products on the pathogenic staphylococcus strains and on the non-pathogenic ones taken from the teguments of the patients was rather weak (± or +).

Thus, the protecting effect of propolis spray cannot be explained by the antibacterial power of propolis *in vitro*.

It is possible that the protecting effect *in vivo* should be due to some mechanisms which differ from those acting *in vitro* and which are not yet known.

TREATMENT OF BEDSORES WITH ANTISEPTIC POWDER WITH PROPOLIS

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The cicatrizing effect of propolis on various wounds and plagues has been reported by numerous research-workers. Even ARISTOTLE showed that propolis cured suppurative sores. PESCHANSKI (1975) and DANILOV (1975) reported good results obtained by treating trophic ulcers of lower extremities with 5% propolis solution; HMELEVSKAIA et al. (1965) reported also good results by treating ulcers following radiation; SUCHY et al. (1975). ZAWADZKI et al. (1975), IOVAN et al. (1975) treated erosions and ulcerations of genital tract in women with good results, BOLSHAKOVA (1975) treated ulcerous varix of shanks caused by burns of the 1st and 2nd grade and by chilblain, POPESCU et al., actinomycosis, ATIASOV et al. (1975) deep burns, MATTEL et al. ulcerous and aphtous stomatites etc.

Starting from these results we decided to study the effect of a powder with propolis on bedsores.

The cutaneous infections and particularly the bedsores take the first place among the complications frequently encountered in bedridden patients in the sections of orthopedy, neurology, psychiatry, geriatry. The decubitus bedsore raises particularly difficult problems even to the hospitals with the most up-to-date equipment for a variety of reasons, namely: the trophic troubles of the background on which they appear, their rapid extension and long lasting development, the danger of superinfection with very virulent germs (piocianic, proteus, pathogenic staphy-

lococcus etc.), the difficulties in manipulating, dressing and taking care of such patients.

Material and method

The preparation was a powder containing 10% propolis and 80% inert siccative powder.

The investigations were conducted on a group A of 12 patients (8 women and 4 men) of different age (between 42 and 86). All of them were bed-ridden by fraction of femoral cervix (9) or by cerebral vascular accidents (3) and showed unique or multiple bedsores in the sacral region. Three of them had also sphincterial incontinence.

They were treated by powdering directly the bedsores once a day with the preparation after previously deterging them with an antiseptic solution (Rivanol). Then the edges of bedsores were sprayed with propolis spray after which a sterile dressing was applied. Sterile dressings were not applied to 4 patients who showed less extensive and more skin-deep bedsores.

Except Rivanol and powder with propolis no other antibiotic or locally or generally cicatrizing medicines were used.

The control group B included 12 patients similar to the experimental one in point of sex, age and morbidity. The bedsores of these patients were deterged with the same Rivanol solution after which they were dressed also once a day with the most effective antibiotic according to the antibiogram.

Both groups received the same general treatment.

To test the antibacterial effect of powder we sowed culture media of gelose-blood with material taken from bedsores at different intervals and tested the sensitiveness of bacterial cultures to the powder. The table given below includes the results of the experiment.

Results

In 9 out of 12 patients of group A the results were very good. The bedsores were cured in a short interval varying from 16 to 54 days depending upon the seriousness of cutaneous lesions and the main affection of the patient.

Bedsores in 3 patients were not cured but they did not aggravate either. They were depressed showing a sphincterial incontinence, serious neuropsychic and trophic cutaneous troubles. In two patients bedsores were infected with *Proteus* which did not disappear until complete cure. No patient showed intolerance phenomena of propolis powder.

As to the control group B bed sores were cured in only 3 patients and in a longer period (43 to 75 days). In 5 other patients bedsores had a longer period of evolution (52 to 86 days), they showed a slight improvement, but they died without being cured. In 4 patients the bedsores aggravated.

With regard to the capacity of inhibiting the *"in vitro"* bacterial cultures we found that propolis powder had no action on *Proteus* and staphylococcus colonies.

	SENSITIVENESS					
	0	±	+	++	+++	Total of cultures
Proteus	21	1	—	—	—	22
Pathogenic staphylococcus 15	15	—	—	—	—	15

0 = resistance
± = 0.5 cm diam. lysis
+ = 1 cm diam. lysis
++ = 1.5 cm diam. lysis
+++ = over 2 cm diam. lysis

Though the number of cases on which the effect of propolis powder was studied was small, the high percentage of total cures (75%) as well as the short time required in comparison with the long period of cures obtained with the group B make us regard the cicatrizing effect of propolis powder as remarkable the more so as this powder was applied once a day and in some cases such dressings were not applied at all.

It is more difficult to explain the inconsistency between the low antibiotic capacity of powder in both *"in vitro"* and *"in vivo"* experiments (the microbial population persisting in the bedsores during all the period of treatment until their healing) and the remarkable cicatrizing capacity of this powder. Perhaps further research on the mechanism of action of propolis on the *"in vivo"* pathogenic microbial flora will bring the solution to this important problem.

These first results of our investigations recommend the propolis powder as an effective therapeutic way of treating bedsores in bedridden patients because it has a remarkable cicatrizing effect, it is easy to use and gives no adverse effects and in the last analysis its cost is rather low in comparison with the antibiotic medication.

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VII. ECONOMIC ASPECTS OF HARVESTING PROPOLIS

SHOULD WE RECOMMEND THE BEEKEEPERS TO HARVEST PROPOLIS ?

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When we raise the problem of the profitability of harvesting propolis, we must first analyse the qualitative and quantitative aspect of this by-product of the hive.

The quality of propolis includes its origin and characteristics.

In point of origin, the propolis is a mixture of different components in different quantities. The main component is constituted by resins which cover the poplar buds and leaves as well as oak-tree, wild chestnut, birch tree ones. These substances are harvested by foragers, especially late in summer and in autumn and are carried on their legs as in the case of pollen. In the hive, this material is taken by the worker bees which after having processed it by help of the mandibles deposit it where necessary.

It is possible that during this processing, some mandibular secretions — maybe of the labial glands — penetrate propolis as it is the case of wax (in order to be better mixed).

Besides, propolis also contains a pollen balm — this is the oily protection layer of the pollen pellets which are easily eliminated in the honey sack. This is why the propolis almost always contains wax in different quantities.

It results that the composition of propolis depends on the plant species visited by bees. The amount of propolis collected depends on the characteristics of the place where bees are located and on the climatic conditions.

The assertion that the active accumulation of propolis by bees at the hive entrance herald a long and hard winter, proved to be often unjustified. However the narrowing of the hive entrance was observed only in hard cold climate zones.

In Vienna for example, bees never reduce even the very large hive entrances of the plastic hives. According to the use of propolis by bees,

one can draw a conclusion as concerns the qualities of this material : it is a building material which isolates water and it is also a device of preservation with antimicrobial characteristic.

The latter is the most important feature.

Of late, these characteristics are under study. Starting from the basic works of BARBIER, GONNET, LAVIE and VILLANUEVA from France which dealt with the antimicrobial effect of flavonoids, POPRAVKO et al. (Soviet Union) extended these researches on bacteria and fungi : by mixing propolis with alcohol, they identified biologically active substances in it.

The propolis preparations are successfully experimented in many clinics, for instance in Romania and in Soviet Union for treating some bacterial, mycotic and viral diseases. Maybe the area of using propolis will grow larger.

The propolis is also a good means of antibacterial preservation for wood and also for varnishing violins. For a couple of years we have been using the acetone extract for permeation of the external walls of the new hives. The substance penetrates the wood. These hives, located in the open, resisted better to bad weather than hives otherwise protected.

The use of propolis for medical and technical purposes depends on the following conditions : first, on the regular delivery of the raw material — which is influenced by the methods of obtaining it and by the climate conditions.

The product with a minimum wax content may be obtained by the deliberate creation of supplimentary bee space about 1—2 mm. Other types of building secure a propolis with a highest content of wax which can be separated by the careful melting of the propolis at the temperature of about 80°C.

The second condition for using propolis especially for therapeutic purposes is its standardization in connection with its biological activity against different or all pathogenic strains of microorganisms.

PRODUCTION OF PROPOLIS

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In the Latvian SSR beekeeping is mainly specialized for pollination of farm crops — mostly red clover seed crops, and honey and wax production. For 10 years, the beekeepers in this country have been increasingly interested in royal jelly, propolis, pollen, and bee bread. Because they carry a small weight in the overall production, mostly amateur beekeepers and a number of beekeepers of the apiaries owned by collective farms have been concerned with them. Obtaining and processing of these products has been coordinated by the branch at Tsessisk of the Society of Horticulture and Apiculture. Also research has been started on use of these products in medicine.

At present, roughly 30 kg royal jelly are produced in the Latvian SSR, 1,700 kg pollen, 100 kg bee bread, and 3—3.5 tons of propolis. The production unit of the experimental laboratory of the Tsessisk branch of the Society of Horticulture and Apiculture produces 11 preparations with propolis. The General Pharmaceutical Direction of the Ministry of Health of the Latvian SSR produces three such preparations, and the Chemical Works at Olain will soon start production of *PROPOSOL.*

Due to the growing interest in propolis and its increasing price, its production is higher with every passing year : in 1971 — 1.5 tons were obtained, in 1972 — 2 tons, in 1973 — 3 tons, and on October 1, 1974 already 2.5 tons were recorded, the average increase of propolis production being of 29%.

The Commission for Prices of the Council of Ministers of the Latvian SSR has fixed the price of one kg of top-quality propolis at 30 roubles, and 10.9 roubles of low-quality propolis.

In one work-day a beekeeper can collect 1—1.5 kg propolis. According to our norms of remuneration, 3—5 roubles are paid to beekeepers for one kg of propolis. Because of the small-scale production, no concern existed for developing eficient methods of obtaining propolis.

Under the conditions in the Latvian SSR, the Caucasian Mountain grey bees and some Migrelian populations are very efficient propolis producers.

Propolis is collected as follows :

(1) By scraping it from frames, hives and the cloth under the cover. Whenever inspecting the nest beekeepers take away the propolis from frames and the hive ; also from the hive from which he moves colonies to desinfected hives ; from the frames when extracting the honey ; and twice during the season — from the cloths under covers. It is a painstaking method, being used mostly by amateurs.

(2) By adjusting frame spacers to the brood frames, which intensifies propolis production ; it is regularly removed by chisel when the brood nest is inspected.

(3) By changing the cloth-tent-cloth or polyethylene fibres.

Cloths are changed twice a year. By this method, good propolis is obtained.

(4) By using a special grate. Beekeeper LEIKART has devised a grate of hard wood, 4×6 mm slats, with 3—4 mm distance between them. Two-three grates are introduced into each colony, and regularly replaced, after which propolis is collected. In practice, all the above methods are used concomitantly.

In considering the productivity of bee colonies in terms of propolis, the following conclusions were reached : every colony, irrespective of race and method of collection, can produce 50—700 g propolis ; with Caucasian Mountain Grey bees, 2—3 times more propolis is obtained. Greater quantities of propolis can also be obtained by intensive ventilation in the brood nest and by improved methods of collection, with due attention being paid to a better remuneration of beekeepers as well.

QUALITY OF PROPOLIS

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USSR

The ever wider and varied use of propolis has called for methods of checking its quality. Only high-quality propolis must reach the collecting centres. So far, no efficient method for accurate checking has been perfected. Because the chemical composition of propolis has been little investigated, it is difficult to ascertain its characteristic features. Also, of the other bee products propolis is quite special having a quite complicated system of components — some of them varying not only according to geographical region but also to season.

The laboratory tests conducted between 1966 and 1969 at the Bee Research Institute have showed that although propolis has a complex, greatly variable composition, all samples contain a specific group of substances which condition physico-chemical and biological features including the antimicrobial ones, which opens up new prospects for its investigation and characterization.

Permanent components in all samples are acid and non-saturated compounds. The iodine number is 44.5 ± 0.89 on an average, ranging between 28.7 and 65.7. The nature of the non-saturated compounds has not yet been identified. But the capacity of propolis has been ascertained — just as royal jelly — of oxidizing with potasium permanganate. (T. V. VAKHONINA, 1968, 1969). One may assess that oxidation of propolis is related to the presence in its composition of a number of non-saturated acids of the fat series with 10 carbon atoms, specific to bee's organism and to glandular secretions, which are included both in royal jelly and propolis.

Consequently, the reaction with potasium permanganate allows not only for qualitative characterization of propolis but also of its origin.

A method of determining the speed of oxidation of propolis and of its extracts has been perfected. The speed of oxidation is expressed by the time (seconds) during which 0.1 ml of the potasium permanganate solution decolorates in a medium which contains the tested product.

Propolis and the dry propolis extracts — in aqueous, alcohol, and ether solutions — decolorate 0.1 ml of potassium permanganate solution. The reaction is instant when the solution contains 1 mg dry substance and more alcohol extract, or 0.1 mg and more aqueous extract. The oxidizing capacity of propolis conditions its antioxidant features.

The speed of oxidation is determined as follows : 200 mg of thinly pounded propolis — weighed very precisely — is introduced into a 250 ml flask, and 5 ml rectified ethyl alcohol is poured on it. After one hour, 100 ml of boiled and then cooled distilled water is added, the mixture being carefully stirred. The solution is then filtered through a paper filter. Into a 150 ml flask 10 ml filtrate are poured, and 90 ml water is added to it. Then 2 ml of the diluted solution is pipetted into a 50 ml glass, 1 ml 20% sulphuric acid is added and the mixture is stirred for one minute. In the acidulated solution, a drop (0.035—0.040

ml) of 0.1 potassium permanganate solution is pipetted, and the fading away of the pink colour of solution is timed. For this test, the temperature of the solution must be 18—22°C.

The method of determining the speed of oxidation of the propolis extracts with suspension of 100 mg dry extract is similar to those already described.

For establishing the speed of acidulation, propolis collected in 1965 in Gorki region was analysed in 1967, as well as its dry extracts — in aqueous, alcohol, and ether solutions (T. V. VAKHONINA, 1972).

With all products tested — which included up to 0.11 mg dry substance of propolis in 1 ml solution — the pink colour faded away in a little over one minute (Table 1). The speed of the reaction depended upon the contents of dry substance in the product tested in solution.

Table 1

OXIDATION OF PROPOLIS AND ITS EXTRACTS

Tested substance	Suspension mg/100 ml	Dilution	Contents of dry substance (mg/ml solution)	Time (sec) of decoloration of 0.1 ml potasium permanganate solution (oxidation coefficient)
Propolis	100	1 : 10	0.75	1.7 (instantly)
Propolis	100	1 : 20	0.40	1.7 (instantly)
Propolis	100	1 : 30	0.24	8.5
Propolis	200	1 : 100	0.18	11.1±0.056
Propolis	100	1 : 60	0.11	70.0
Propolis	100	1 : 20	0.08	Does not decolorate
Alcohol extract	100	1 : 40	0.20	11.0±0.68
Alcohol extract	100	1 : 10	0.09	21.0
Alcohol extract after removal of aqueous extract	100	1 : 10	0.09	6.16±0.08
Ether extract	100	1 : 10	0.11	Instantly
Acetone extract	100	1 : 10	0.60	Instantly
Aqueous extract	100	1 : 10	0.09	Instantly
Control (medium without propolis)	—	—	—	Does not decolorate

The solution decolorated in 11.1±0.056 seconds — when it contained 0.18 mg propolis dry substance. When the solution contained less than 0.18 mg propolis dry substance, the colour would fade away within longer time. When the suspension contained a double quantity of propolis dry substance, the solution would decolorate instantly.

The most active were the extracts in ether, acetone, alcohol, and above all the aqueous propolis extract. The permanganate solution in medium of aqueous propolis extract — 0.09 mg dry substance in 1 ml — decolorated instantly or in 5.0—18.0 seconds; in medium of alcoholic extract — 0.18—0.20 mg dry substance — in 11.1—11.0 seconds.

The activity of extracts was found to depend on the temperature at which extraction was made (Table 2). The speed of oxidation is higher

Table 2

SPEED OF OXIDATION OF AQUEOUS EXTRACTS

Locality of origin of propolis from which extracts were obtained	Temperatures at which extracts were obtained	Pink colour disappears in ..... (sec)
Yaroslavsk, the regional beekeeping centre	22	19.5±0.5
	40	13.5±0.5
	70	10.5±0.6
	90	Instantly
Voronej region, Petropavlovsk district, „Trud" Kolkhoz	22	17.5±0.6
	40	10.5±0.6
	70	4.5±0.6
	90	12.5±0.6
Novosibirsk region, Cherepanovski district, Murygino	22	15.5±0.6
	40	6.5±0.6
	70	9.5±0.6
	90	12.5±0.6

Note : dry extract suspension — 100 mg ; dilution 1 : 10

with the aqueous extracts obtained at high temperatures — 40°, 70°, and 90°C. With the alcohol extracts, and alcohol extracts after separation of the aqueous fraction — an inverse dependence was recorded : the speed of acidulation is lower for the alcohol extracts obtained at 70°C as against the alcohol extracts obtained at 22°C.

Hence the propolis fraction soluble in water has superior qualities as compared with the alcohol, acetone, and ether fractions, and with propolis in its initial condition.

This was also confirmed by the existence of the antimicrobial features. It results that the speed of oxidation allows for more thorough knowledge of the quality of propolis than the iodine number, and can be used as indicator of purity of the product.

Neither beeswax nor the propolis fraction insoluble in alcohol-ether mixture have the antioxidant features specific to propolis. In fact they have no antibacterial features. Because in the insoluble fraction of propolis — beeswax in it included, the quantity of active substances is insignificant, only very great quantities of suspension a decoloration reaction of the potasium permanganate solution takes place (Table 3).

Table 3

PROPOLIS IMPURITIES AND THE OXIDATION REACTION (FOR 200 mg SUSPENSION)

Name	Dilution	Contents of dry substance (mg/ml theoretically)	Pink colour disappears in ..... (sec)
Beeswax	1 : 50	0.18	Does not decolorate
Beeswax	1 : 5	2.0	9.2±0.26
Insoluble fraction	1 : 50	0.18	Does not decolorate
Insoluble fraction	1 : 5	2.0	6.1±0.13

Note : dry extract suspension — 100 mg ; dilution — 1 : 10

Indeed, the permanganate solution decolorates in the presence of a quantity of 2 g propolis beeswax or of insoluble substances with mechanical impurities in 1 ml of solution. 0.18 mg/l ml is the quantity for propolis with standard composition.

Consequently, beeswax and the insoluble fractions of propolis do not decolorate the potassium permanganate solution under the existing conditions, and do not have antioxidant features ; on the contrary, they diminish such features in propolis.

With the propolis with a higher content of mechanical impurities (that collected from the cloth beneath the inner cover) the speed of oxidation is low — the solution decolorates in 17.5 seconds, while the relatively pure propolis collected from the bars and hive walls is more active — the potassium permanganate solution decolorates in 6.5 seconds, and with the propolis collected from the hive entrance — in 4.5 seconds.

It results that in the impure propolis the content of active substance declines, fact revealed by the reaction of decoloration of the permanganate added to the solution with the tested substance. The data in tables 4 and 6 refer to the propolis collected at the apiary of the Bee Research Institute, Ryazan region, in 1968, from Central Russian bees, which was tested in the same year. The test was made with 0.18 mg dry substance in 1 ml, 200 mg propolis, and 1 : 50 dilution.

The speed of oxidation depends on the quantity of beeswax in propolis (Table 4). It increases with the increase of the quantity of beeswax in propolis.

Table 4

SPEED OF OXIDATION AND BEESWAX CONTENTS OF PROPOLIS

Locality where propolis was collected	Beeswax contents of propolis, %	Oxidation coefficient (sec)
Novosibirsk region, Cherepanovski district, Karasevski People's Council May-June, 1968	31.6	20.5±0.5
Moscow region, Luhovitski district, August-September, 1968	28.1	18.5±0.5
Vladimir region, Yuriev-Polski district, „Riabininski" sovkhoz August-September, 1968	7.77	14.5±0.5
Ryazan region, Rybnoe district, August-September 1968	5.20	12.5±0.5

As beeswax and mechanical impurities determine the quality of propolis, and the speed of oxidation varies according to the content of wax and mechanical impurities, the quality of the product can be deduced after the speed of oxidation.

Table 5

OXIDATION COEFFICIENT OF PROPOLIS OF VARIOUS ZONES

Zone	Oxidation coefficient (sec)
IInd forest and pasture (clover and rasp. berry)	12.5 ±1.5 (5.0—20.0)
IIIrd forest and plane (buckwheat and lime-buckwheat)	14.88±1.78(6.0—25.0)
IV-Vth steppe, slyvosteppe (sunflower, coriander)	14.9 ±2.9 (4.0—24.0)
IXth sylvosteppe (Western Siberia)	16.6±0.8 (14.0—21.0)

The antioxidant features of 50 individual samples from 24 regions of the Russian SFSR were tested. The most active was found to be fresh propolis — up to 1 year ; the time of decoloration of the potassium permanganate solution was 9.38±0.72 seconds as against the average of 11.1±0.056 seconds.

The oxidizing capacity is specific to all individual samples of propolis from various regions of the Russian SFSR. Although the oxidation coefficient does not have the same value, it ranges between 14.9±1.02

Table 6

OXIDATION OF PROPOLIS FROM TWO BEE RACES

Place and period of collecting	Pink colour disappears in . . . (sec)	
	Central Russian bees	Mountain Grey Caucasian bees
Frame bars, May-June		4.5±0.55
Frame bars, July-August		5.5±0.50
Frame bars, May-June	7.5±0.55	
Frame bars, July-August	7.5±0.55	
Cloth, July-August		5.5±0.5
Cloth, July-August	18.5±0.55	
Cloth, September	11.5±0.50	

seconds. The limits according to zones range between 12.5±1.5 and 16.6±0.8 ; significance — 95.5% ; the limits according to samples range between 4.0 and 25.0.

No significant difference was recorded between the speed of oxidation of the propolis collected from various climatic zones, except the propolis from the IXth zone of Western Siberia. The antioxidant features of the propolis from that zone are relatively weak, but not below the standard. Its aqueous extract has a low iodine number as compared to the propolis from the European part of the Russian SFSR (the IV—Vth zones).

Also investigated was the propolis collected from colonies of two races, developed under the same conditions (Ryazan region, Rybnoe apiary of the Bee Research Institute, 1968). The difference between the speed of oxidation in propolis samples from the two races was insignificant: Central Russian — 7.5 seconds; Mountain Grey Caucasian — 4.5—5.5 seconds.

The period of storage of propolis under certain conditions had no significant influence on its features. When stored at room temperature for 3 years, the speed of oxidation has not changed, or increased by 1—2 seconds.

The speed of oxidation of propolis depends on the storage conditions: at room temperature it is higher than when it is stored in refrigerator (—4°C). The difference recorded ranged between 2 and 6 seconds, and according to samples — between 10 and 11 seconds. Similar results were obtained with the propolis collected from various climatic zones of the Russian SFSR.

Thus, a method of determining the speed of oxidation of propolis and of its aqueous, alcohol, ether, and acetone fractions was developed, as indicator of the quality of propolis.

The speed of oxidation of propolis depends on the content of dry substance of the product investigated in solution. The most active were found to be the ether, alcohol, acetone, and above all the aqueous extracts

Beeswax and the fraction with mechanical impurities insoluble in water — in alcohol — ether and acetone — do not have the antioxidant features specific to propolis.

The speed of oxidation varies according to the quantity of beeswax and of insoluble substances in propolis and, to a certain extent, according to the geographical origin of the product.

The antioxidant features of propolis do not change significantly after 3-year storage.

The speed of oxidation allows for identification of its origin.

METHODS OF OBTAINING PROPOLIS

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The Symposium *) is intended for approaching propolis in terms of chemical composition, its biological and pharmacological effects, and its use in medicine and other domains.

Essential for using propolis for these purposes is to obtain enough propolis of known origin.

In the beekeeping practice at present, propolis is a substance which renders handling of frames more difficult, stains hands, clothes, and

*) First International Symposium on Propolis, Bratislava, November 1972.

often causes allergies. Propolis is removed from frames and other hive parts only when used for production. When careless handling results in its being mixed with wax, the quality of the latter is altered, and foundations are easily breakable.

By an adequate method, a great quantity of propolis can be obtained even from a small colony. Most often, its collection is accidental, irrational, primitive, and difficult. The substance obtained is not pure, its origin and time of production by the colony are not known, and its non-standard properties either. The literature available does not mention any method of rational collection of propolis, and propolis is not used for other purposes.

The Bee Research Institute at Dol is concerned with systematic possibilities of using all bee products; as this year propolis is in increased demand, the Institute has suggested once again — jointly with the Centre for Inventions and Improvements — to undertake study on "methods of obtaining propolis" as nobody has approached them yet.

In order to be able to investigate the chemical composition, effect, and use of propolis — which is a vast domain — specialists should first make a thorough and precise study of apiculture — as it is the key to obtaining great quantities of top quality propolis. Therefore, we consider the apicultural specialists should focus their effort on:

1. Collection and thorough study of the origin of propolis components, the way in which it is collected, and its storage by bees in the hive;

2. Collection and thorough study of the part played by bees glands to collection, storage, moving by bees, and use of propolis;

3. Efficient use of the knowledge of propolis — producing impulse of bees, namely providing of conditions which should stimulate bees to store a great quantity of propolis in certain places in the hive, possibly in special devices enabling hygienic and easy collection of propolis;

4. Development of a method of obtaining a great production of propolis, by making use of the race characteristics of the honey bee, and by selection on purpose;

5. Promotion of and providing for the necessary conditions for adequate collection and organised purchase of propolis from experienced beekeepers at rewarding prices;

6. Development of new methods of specialized collection of the propolis having specific characteristic features, in specific regions and periods of time, on the basis of the knowledge resulting from thorough research on propolis, and with due account being taken of the specific requirements concerning acknowledgement of its effects;

7. Development of a method of obtaining propolis — or of separating propolis from wax;

8. Development of adequate methods of processing raw propolis according to the purposes it is to be used for.

The major concern is development and perfection of methods of collection of propolis. Now propolis is scratched from frames, gaps, inner

covers, frame bars and more seldom from around the entrance. The attempts to direct the propolis-producing impulse up to now included removal of the inner cover, introduction of wire or plastic grates, or by providing for carvings in the wood bars and other parts of the hive. In our experiments, we have been striving to retain propolis in the plastic mesh of the grates wherefrom it can be collected more readily and hygienically, by removal from the frame. We point out that in practice difficulties arouse because of smaller production of propolis which is accounted for by the fact that the stock of the Institute apiary was completely replaced by carniolan bees, Troiseck strain.

Allow me to make several remarks and recommendations which — under the present requirements — I consider important in devising and improving methods of obtaining propolis.

1. An essential turning point in beekeeping was due to the genial discovery of Langstroth who in 1851 determined the room where bees do not store propolis and wax either — 3/8—3/16 inch (4.7625—9.5250 mm). Narrower intervals, through which bees can not pass, are all plugged with various materials, propolis included. We point out that at present the limit interval is 4.5—4.8 possibly 5 mm. But no one knows how deep is propolis stored in narrow intervals.

It is very likely that the limit of interval and depth depend on geographical region.

2. The assessment that propolization is the result of the bees self-defence against cold, wind gusts and draught is, at least in some cases, wrong. More acceptable is the assumption that bees defend themselves from pests and intruders by sealing the intervals through which they cannot pass and by erecting protecting walls at the entrance. The impulse of propolization is manifest to a less or greater extent on all objects in the hive, combs and diafragms included. It is quite possible we do not know the precise and real reason as yet.

3. The natural sources of propolis are of two essentially different kinds : the first are the resin substances — most of them collected by bees from plant buds with their mandibles and carried in the baskets on their hind legs. The sources include poplar *(Populus)*, chestnut tree *(Aesculus hippocastanum)*, birch tree *(Betula)*, alder tree *(Alnus)*, spruce fir *(Picea)*, pine tree *(Pinus)*, ash tree *(Fraxinus)*, cherry tree *(Prunus avium)*, plum tree *(Prunus domestica)*, fir tree *(Abies)*, sunflower *(Helianthus)*, willow *(Salix)*, elm tree *(Ulmus)*, oak tree *(Quercus)*.

The second group includes the balsam in pollen, released during the breaking and processing of pollen grains from entomophilous plants, — from their oily skin ; when the sac is full, the balsam is pushed by the proventriculus valves. It is spread by bees, with their proboscis, all over the objects around the brood. During the processing of resins by mandibles, secretions of the workers mandibular glands also mix with them. The colouring in yellow of pharyngeal glands in worker bees experimentally fed on albumin attests to the possibility of the glands contributing to the colouring of the surface of objects inside the hive.

4. The great number and various origins of sources of the components of propolis provide for a considerably wide range of possible composition and consequently also of effects of propolis ; this means that standard kinds will be obtainable only by thoroughly devised methods of collection. Solution must also be found to transportation and blending of the propolis collected earlier with the fresh one. All this allows us to understand the difficulties to be faced in meeting the standard requirements for the desired efficient substances.

5. Propolis and wax are very seldom used as such, because in propolizing bees substitute wax for propolis. Therefore, propolis contains variable amounts of wax. By adequate methods of separation top-quality wax and pure propolis can be obtained.

6. Various opinions exist with respect to pollen balsam being or not used, together with resins, in preparing the propolis existing in the hive. Some authors hold that bees propolize mostly in August, September and October — when the period of abundant pollen is over. Also doubtful is the possibility of obtaining a greater quantity of propolis from the small amounts of pollen pellets. Experiments have beeen done for recording propolization in caged bee colonies. By such an experiment McGREGOR found in 1952 that bees are not capable of gathering propolis when pollen lacks in their food ;

I myself consider that pollen balsam is a component part of propolis.

7. The only source of reference which I have consulted mentioned the average annual production of propolis of 5 bee colonies (with race not being specified) — roughly 65 g/colony. Certainly, the fact that propolis is dispersed throughout the hive is a factor limiting its collection.

The factors limiting propolis production include :

a. The small number of constant gatherers of propolis (only several tens in a colony).

b. Discontinuation of propolis collection during abundant honey flows.

c. Often interruptions in propolis processing when foragers fly back to the hive to store their loads.

d. The impossibility of unloading pellets without the help of other bees or without lower temperature.

e. Limited collection and storage of propolis due to oscillation of environment temperature.

8. As substitutes of resins, the following, more unusual materials are used : tar, asphalt, liquid used for impregnating wood, grafting wax, varnishes, plant waxes, and synthetic waxes.

9. In the Czechoslovak Socialist Republic, *A. m. carnica* bees are most widely spread ; it produces less propolis than our native bee. Under the conditions in this country, *A. m. caucasica* produces more propolis, especially on very large areas on the side walls of the universal Morav hive. Also the African bees — *A. m. unicolor* of Mada-

gascar and *A. m. intermissa* are rated as intensive propolizers. Considering the conditions in Czechoslovakia, the propolization impulse will be promoted by planned breeding of pure race bees or by crosses ; also, lines of bees are envisaged to be developed for specific sources of propolis.

This report was meant to make known to the participants and especially to beekeepers the conditions of propolis production at present and methods of obtaining the desired qualities and quantities of propolis.

In my opinion, the growing interest taken in propolis will contribute to understanding the need to set up a permanent working group — to include representative of all domains related to the study of propolis ; the working group should collect all relevant information and coordinate the requirements of various sectors of activity.

Any suggestion in this respect is welcome.

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