

## CONTENTS

[Higher Plants](#)

[Lower Plants](#)

[Transgenic Plants](#)

[Fish](#)

[Yeast](#)

[Poultry](#)

[Veterinary](#)

[Medicine](#)

[Bacteria](#)

## Higher Plants



Background of the problem. The origin and the evolution of life on the Earth were always accompanied by influence of exogenous electromagnetic fields. The sun is the most powerful natural source of electromagnetic fields; it forms the natural electromagnetic background in biosphere. However, the sun's radiation in millimeter band (extremely high frequency - EHF) is almost completely absorbed in high atmosphere layers (mostly by water steam) causing minimal electromagnetic background ( $10^{-21}$  watts/m<sup>2</sup>), although there are some thin transmission lines of diapason. Obviously, the every part of the electromagnetic spectrum contributed to evolutionary process.

Any bio-system is bound to as to external, and to inner electromagnetic fields. According to the scientific data homeostasis of living system is concerned with emitting of electromagnetic fields by cells exactly in millimeter band of spectrum; the parameters of this radiation are sensitive indicators of physiological condition of organism. There are a lot of sources of mm-wave radiation in biological tissues; they rapidly attenuate but have a synchronizing influence on nearest generators, which also emit mm -waves, etc. Probably, these radiations concerned with membrane structures of cells and biologically important macromolecules. The fitness of organism's radiation with atmosphere absorption bands of mm -wave radiation creates the most favorable conditions for appearance and development of own informational - wave systems, since there appears an ability to realize synchronization in bio-systems using low-level control signals.

The problem of interaction between low-intensive exogenous electromagnetic fields and biological objects is fundamental for science and practice now. Water plays an important role in this interaction; it is a basic component of all living systems, it constitutes 65-98 % of organism's mass, and also acts as wideband absorber of mm-wave radiation. It is shown, that a water layer of 1 mm thick attenuates incident radiation of 8 mm wavelength by 100 times, with 2 mm wavelength – by 10000 times. It is considered that extremely a high frequency radiation almost entirely attenuates in tectorial tissues of organism at depth of 0,5 – 0,7 mm. The great biological effect of extremely high frequency radiation, that was observed nevertheless, is probably caused by special resonance state of water. A phenomenon of resonance transparency of water was detected at certain frequencies. It was noticed that water “respond” for extremely high frequency radiation is reached on frequencies that are not matching the acting ones. A “memory of water” on previous exposure of electromagnetic waves was revealed. Obviously, molecular oscillators of water component of living organism, self-synchronizing on resonance frequencies, represent natural inner source and conductor of mm-waves. However, there are a lot of unknown in mechanisms of action of extremely high frequency radiation on biological system.

The application of microwaves in agriculture and biotechnologies is coming more perspective now. There is a reason to suppose, that a purposeful action of high frequency therapy radiations on organism will allow to manage many of vital functions, to influence on many parameters of growth and development of plants and animals, to cure diseases, etc. Our scientists in their pioneer works with photosynthesizing objects (cyano-bacteria, algae, cucumber germs) revealed an important role of ultra high frequency radiations in plant cultivation: an increase of indicators of growth, of biomass, of quantity of pigments, of excretion of organic compounds is registered, an optimization of photo-breath, bioelectrical activity, intensity of absorption (secretion) of ions  $K^+$ ,  $Na^+$ ,  $Cl^-$  etc. is indicated. As a result original conceptions of reasons of forming stimulating effect of microwaves on photosynthesizing organisms are developed. Thus, the beginning of developing of important for mankind new directions in the fields of informative mm-wave band application - in agriculture and the biotechnologies.

major properties of biosystem reactions on mm-wave radiation:

Objects of research	Objects of mm-wave radiation	Expected results	Applications
Higher plants (wheat, barley, pea, sea-buckthorn, apple-tree, currants, etc.)	Seeds, germs	Germination, growth, vital functions stimulation	<ol style="list-style-type: none"> <li>1. Plant cultivating: germination, growth, resistance, productivity stimulation.</li> <li>2. Brewing: wort production effectiveness increase.</li> <li>3. Food industry: biologically active admixture production effectiveness increase.</li> <li>4. Pharmacology: biologically active compounds production effectiveness increases.</li> </ol>
	Cell and tissue cultures		
	Water and liquors		
	Fruits, vegetables, roots, flowers	Quality improvement, shelf life increase	
Lower plants (mushrooms, algae)	Lower plants	Vital functions stimulation, productivity increase	<ol style="list-style-type: none"> <li>1. Mushrooms cultivating: productivity increase.</li> <li>2. Food industry: effectiveness of algae usage increase.</li> </ol>
Microorganisms (yeast, bacteria)	Microorganisms	Vital functions stimulation, productivity increase	Production effectiveness increase: <ol style="list-style-type: none"> <li>1. cheese-making;</li> </ol>

			<ol style="list-style-type: none"> <li>2. baking of bread;</li> <li>3. dairy production;</li> <li>4. wine-making;</li> <li>5. forage albumen;</li> <li>6. amino acid, vitamins, etc.</li> </ol>
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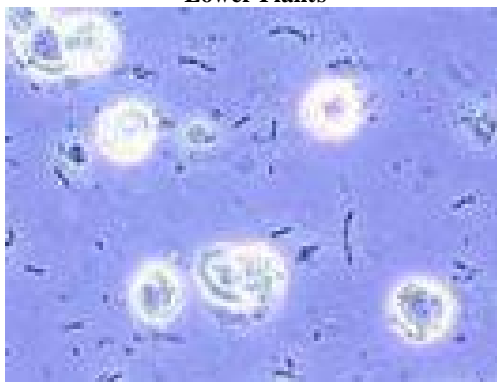
Objects of research	Objects of ultra high frequency radiation	Expected results	Applications
Vertebrate (cows, horses, calf, dogs, cats)	Breast, uterus mucosa, etc.	Sanative effect	Veterinary medicine: <ol style="list-style-type: none"> <li>1. Mastitis curing (cows).</li> <li>2. Endometritis curing(cows).</li> <li>3. Pneumonia curing (calf).</li> <li>4. Myositis, arthritis, enterocolitis, emphysema, overfatigue curing (horses).</li> </ol>

Insects (bees, etc.)	Pupae, larvae, imago	Processes of growth and development management	<ol style="list-style-type: none"> <li>1. Food industry: safety of food from insects.</li> <li>2. Beekeeping: productivity increase.</li> </ol>
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General scheme of study is viewed below:

mm-wave radiation				
Water		Subject of study		Testing conditions
Plants (barley)				
Morphophysiology, biochemical and microbiological characteristics				
Experimental data analysis				
Suggestions for manufacturing				

## Lower Plants



Mm-wave influence is observed on various microorganism taxons. Bactericidal impact of mm -waves, their influence on microorganism reproduction and different substances synthesis proves mm -waves influence on plant or animal cell vital functions.

Biologically active for microorganisms wave lengths spectrum from 4,11mm to 8,34 mm and allow to reveal similarity and difference of biological effects at microorganism exposed by mm waves. Let's recite number of similar characteristics in reaction to mm wave of microorganisms capable and incapable of photosynthesis. Similarity is revealed in many cases when irradiation was performed once. Intensification of growth and biomass outcome was observed as well as resonance effect under irradiation influence, biological effect dependence on parameters of irradiation and, mainly, on wave length, growth inhibition or absence of effect when optimal wave length is changed; negligible quantity of absorbed energy when objects are irradiated; changing of cell membrane permeability; changes in synthesis of biologically active compounds; dependence of received effect on incident power density; dependence of active wave length resonant peaks on genus, species and culture of the microorganism, i.e. individual taxonomic sensitivity to mm wave exposure; absence of exposure mutagenic effect; prolongation of stimulating effect when passaging cultures afterwards - all these phenomena were observed. There were also revealed some differences in microorganism response to mm wave exposure:

1. With nonphotosynthesizing microorganisms, especially yeast, in some cases for obtaining well-defined stimulating effect there was necessary to apply repeated irradiation, such cases were not observed with photosynthesizing microorganisms;
2. With nonphotosynthesizing microorganisms, especially yeast and mushrooms, undergo cell structural changes, which doesn't occur with photosynthesizing microorganisms;
3. Difference in the range of active wave length (photosynthesizing microorganisms have shift toward 10mm);
4. In some cases mm wave bactericidal action on nonphotosynthesizing microorganisms was observed.

Recently growing attention is paid to studying question concerning technique of millimeter wave interaction with biological objects, including microorganisms. In particular, many scientists took interest in how electromagnetic radiation can influence the processes of light energy photosynthetic conversion which proceed in microorganisms. So, the results of mm wave exposure on dynamics of triplet states formation photosynthetic reaction centers of purple bacteria were published [74]. The researches were performed on photosynthetic membranes and on isolated from the membranes reaction centers of Rhodobacter sphaeroides bacteria. As a result the possibility of nonthermal intensity mm wave exposure (4,04mm,  $P=30 \text{ mWatt/sm}^2$ ) under continuous impact on dynamics of singlet -activated primary ion-radical pair of reaction centers transition into triplet state was revealed. Millimeter wave exposure influences also the processes of further decontamination of triplet states in reaction center with carotinoid molecule participation. It can be observed that life of carotinoid triplet increases considerably (almost twice as much) under UHF-radiation influence [72, 73]. It was also revealed that UHF -radiation stimulates photoinduced intra-albuminous electron transfer in quinine acceptor system in reaction center of photosynthesizing bacteria Rhodobacter sphaeroides [74].

By means of impulse spectroscopy method UHF -radiation influence (wave length - 4,04 mm, incident power density -  $30 \text{ mWtt/sm}^2$ , duration of single impact - 10 min) on characteristics of light induced processes in chlorophyll-containing proteins of bacterial photosynthetic reaction centers and on dark

transmutation kinetics of bacteriorhodopsin retinene -containing protein in purple membranes of halobacteria Halobacterium halobium was studied [74]. The authors revealed that UHF -radiation speeds up proton transfer at the regeneration stage of initial bacteriorhodopsin pigment form by 30%.

It is important to note that among the researches on mm wave effect upon microorganisms there are some investigations, which do contain reliable data proving clear difference between radiated and irradiated cultures. Such outcome was received in studies on Saccharomyces cerevisiae yeast. In that case yeast culture was put under mm wave influence with wave length 7,17...7,21 mm, remarkable difference was not obtained, which might be explained by the fact, that there is no active frequencies in the mentioned frequency band [75]. Statistically significant changes of colicin induction, proliferation, membrane state in cells E.coli were not achieved [76]. The reason was, obviously, the same: optimal radiation parameters for the definite object were not selected.

It is also necessary to mention that, unfortunately, some authors in their articles do not always describe in detail all the conditions of conducting the experiment (wave length, capacity, etc.), which makes it difficult to interpret the results. For obtaining well -defined stimulating effect of mm wave influence, as it has been proved by our experience, it is necessary to fulfill time -consuming work on optimal irradiation parameters selecting; while using of arbitrarily chosen characteristics, which can be observed in a number of works, probably, leads to achieving negative results.

The data given below show distinctly that mm wave exposure influences wide range of microorganism properties. The authors also report that mm wave impact has distinct resonant character: there is an individual wave length or wave lengths, which provide maximal mm wave effect for separate organism and for different characteristics of metabolism. Besides, it was revealed that the less is vital activity of an organism, the more effective is mm wave exposure [14]. All the data from this review are summarized in table 1. There are available mechanisms of mm wave influence on photosynthesizing suggested in the last years' surveys [46, 47, 48, 49, 50]. The specified model of probable mechanism for mm wave effect is shown in Picture 1.

Table 1

MM wave exposure effect on different taxonomic groups of microorganisms

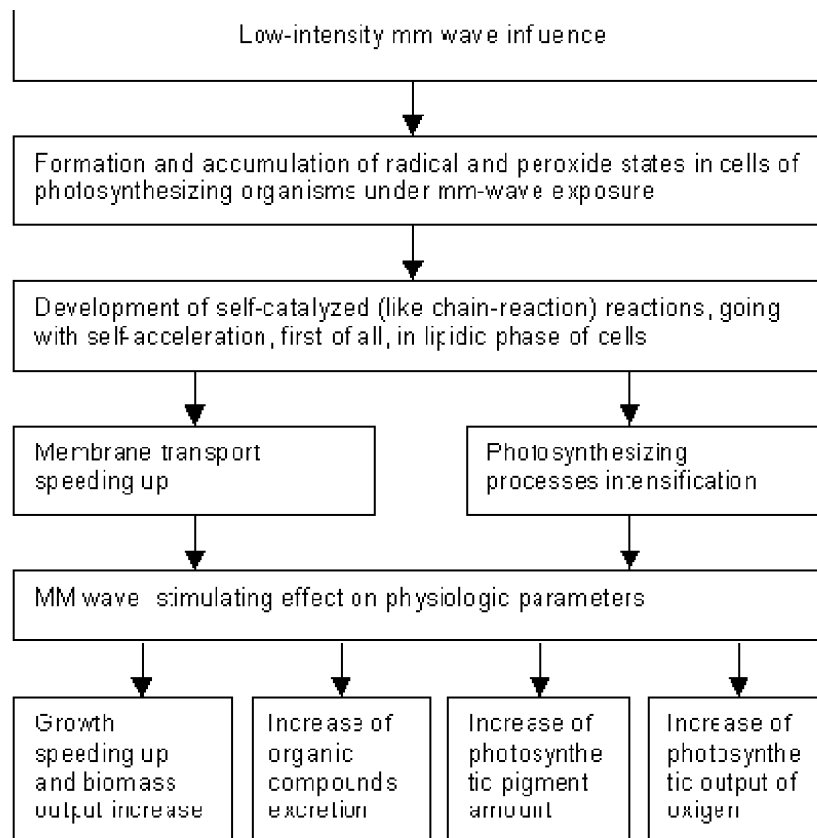
Active wave lengths (mm)	Microorganisms	Radiation effects	References
	Prokaryotes		
	Bacteria		
6,0-6,7; 7,1; 5,6; 5,95-7,2	Escherichia coli	Growth stimulation; Increase of resistance to dehydration	[5]; [17]; [18]; [9]; [10]; [19]; [21]; [22]; [20]
6,0-6,7; 8,0 5,95-7,2	Staphylococcus aureus Staphylococcus sp.	pigmentation diminution; colony size decrease	[17]; [18]; [36]; [37]; [23]; [13]; [14]; [11]; [19]; [21]; [22]
5,95-7,2	Bacillus mucilaginosus	Activation of biosynthetic processes	[36]; [37];
5,95-7,2 6,2	Bacillus firmus	Enzymatic activity and biomass accumulation increase	[14]; [35]; [36]; [37]
8,0	Sulmonella typhimurium	Free radical reactions activation	[69]; [13]
4,04	Rhodobacter sphaeroides	Increase of reaction center triplet states output; triplet life time increase	[72]; [73]
4,04	Halobacterium halobium	Proton transfer increase	[73]
6,96; 4,16; 7,1; 4,46	Photobacterium leiognathi	Bioluminescence quenching; Bioluminescence activation	[38]; [39]
	Actinomycete		
5,95-7,2	Streptomyces spheroides	Increase of ferment biosynthesis activity	[14]; [35]; [36]; [37]
5,6; 4,6	Streptomyces xanthochromogenes	Speeding up of colony growth	[40]
5,95-7,2	Nocardia sp.	Increase of ferment biosynthesis activity	[14]; [36]; [37]
	Cyanobacteria		
6,06; 6,25; 6,66; 7,1; 7,89; 8,34; 4,6; 5,6;	Spirulina platensis Spirulina maxima	Growth stimulation; Photosynthesis intensification	[46]; [61]; [70]; [44]; [49]; [50]; [47]; [48]; [52]; [53]; [45]
8,34	Anacystis nidulans Anabaena variabilis Plectonema boryanum Fremyella diplosiphon	Growth stimulation	[41]
	Eukaryotes		
	Mold fungi		
5,95-7,2	Aspergillus oryzae	Increase of biomass accumulation and ferment biosynthesis	[58]; [57]; [6]; [14]; [36]; [37]
5,95-7,2	Aspergillus awamory	Diminution of pigmentation and conidium formation;	[6]; [14]; [36]; [37]
	Yeast-like fungi		
5,95-7,2	Endomyces fibuliger	Stimulation of yeast cell formation; Increase of glucoptyalin and alphaptyalin activity	[14]; [36]; [37]
	Microfil fungi		
5,95-7,2	Dacthilyum dendraides	Increase of protease activity	[36]; [37]
	Yeast		



8,5; 7,17-7,21; 5,95-7,2	<i>Saccharomyces cerevisiae</i>	Stimulation of growth stimulation biosynthesis activity	[6]; [31]; [27]; [36]; [37]; [74]
5,95-7,2; 6,035	<i>Saccharomyces Carlsbergensis</i> ( <i>pastorianus</i> )	Development speeding up; time discreteness	[6]; [27]; [24]; [35]; [33]; [26]; [15]; [14]; [34]; [16]; [36]; [37]
	Algae		
7,1; 5,6	<i>Scenedesmus quadricauda</i>	Environmental toxicity decrease	[70]; [46]; [44]; [47]; [48]; [49]; [50]
6,06; 7,1; 8,34	<i>Platymonas viridis</i>	Growth stimulation; Photosynthesis intensification	[55]; [56]

Picture 1

Available mechanism of mm wave exposure on vital activity process of photosynthesizing microorganisms



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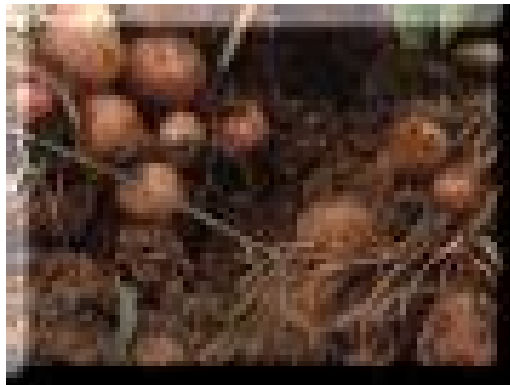
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## Transgenic Plants



### Urgency of the Problem.

Nowadays electromagnetic radiation of MM wave band (MM waves) is widely used in various branches including those, which requires plant productivity increase. There is number of works reporting about positive influence of MM waves to growth of normal plants. However, there are no works on MM wave influence on genetically modified plants. One can suggest that irradiation of transgenic plants by MM waves will allow to ground ways of improve productivity of genetically modified plants. Productivity effect can be achieved both for general increase in plant's biomass and for specific output of recombinant products. Recombinant proteins can be referred as recombinant products, its genes are built -in into plant to acquire bioactive substances or vegetable vaccine. But to present day no scientific study has been performed on MM wave influence to cloning efficiency of genetically modified plants, recombinant protein production in transgenic plant and biomass increase.

It is known that plant systems ensure effective expression and post translating processing (glycosylation, phosphorylation, correct folding and self-assembly) for initial protein products which are important biomedicine preparations. One of trends for biomedical application of plant-based is producing of "eatable vaccine", which gives basis for wide and effective immunization for population and domestic animals [25, 9].

However, relatively low expression levels of foreign antigens synthesized in transgenic plants, coupled with the modest immune response following oral immunization, remain limiting factors for development of an effective plant-based vaccine [35]. Yet, to produce an appropriate immune response in case of oral use of modified plant relatively large doses of antigen are required. In this connection the problem of increase for antigen expression in transgenic plant producing recombinant components of vaccine is of great importance.

There is one more actual aspect of suggested study. It is assumed to use model of newly originated transgenic plant modified by group A rotavirus genes. Group A rotaviruses are significant cause of neonatal diarrhea in humans and several animals species, including calves [21, 34].

The planned study involves investigation of MM wave influence on efficiency of cloning for genetically modified plants on the basis of newly originated model - clover transgenic plant, modified with human and calf rotaviruses, expression for induced genes and optimal operating parameters, allowing to ensure increased output for protein product. The reveal of positive influence of MM wave radiation on transgenic plants efficiency, biomass increase or recombinant products output will open new opportunities for microwave application both in genetic engineering and in cultivating of genetically modified plants for its productivity growth.

State of the question.

By now the main trend in study of MM wave application for planting is seed's preplant processing. There are a considerable number of publications on the topic. Received data testifies to positive influence of MM wave on plant's growth. It is established that preplant processing significantly increases germinating power of seeds [2]. Another works revealed increase for biomass growth for vegetative sets and fruits (or seeds) upon seed processing by MM waves [33, 29].

Irradiation of grown plants is illustrated MM wave influence on photosynthesis reactions. Besides, MM wave irradiation had a statistically valid decrease for thermoinduced inactivation for functional characteristics of photosynthesing apparatus [23]. Mechanism of MM wave interaction with living cell is not yet studied. But suggested model comprises the following factors:

- Resonance and heat surface effects,
- Changes in water structuredness,
- Ion redistribution, which causes increase in  $\text{Ca}^{2+}$  concentration, which in its turn leads to activation of fermentative system and redistribution in potentials at inner and inter cell membranes [2].

The said changes resulted to cell mitosis synchronization. It is also reported about positive MM wave influence to

synchronization in cell mitosis in germinal meristem for germ of cereal crops [33].

In observed references there were no any reports on investigations of MM wave influence on genetically modified plants. No data about MM wave influence on cloning efficiency using apical meristem cells, on biomass for transgenic plants. There is one more important point - about MM wave influence on transgenic plants production of recombinant proteins. Transgenic plants are widely spread in modern agriculture targeted to obtain resistivity against plant pests, to raise plant's productivity and to low production cost and also for many other purposes. One of important trends is application genetically modified plant for new type of vaccine. Genetically modified plants can be used for recombinant protein accumulation, rendering several proteins of infection pathogen with its further cleaning and building-in into recombinant vaccine complex [30]. Such accumulation of recombinant protein is more effective in comparison to existing technologies on E. coli and yeast protein accumulation. There is one more opportunity to use genetically modified plants in vaccine programs - creation of mucositis vaccine for oral use. Such vaccines can be formed basing on non-fractional genetically modified parts of plant. Application of mucositis vaccine is more preferable at prophylactic of infections caused by agents penetrating into the organism through mucous tunics, for instance for enteric infection prophylactic. Rotavirus infection is one of the main enteric infections.

Rotaviruses (Reoviridae, Rotavirus) - are the main pathogen for acute diarrhea disease. The disease is occurs for children at the age up to 3 years and for people above 60 years [14] and one of the reasons for restraining cattle stock increase [34].

Great benefits for world community were connected with tetravalent reassortant vaccine RRV-TV (commercial title Rotaschield), investigated at National Health Institute USA [12,14]. But now usage of the vaccine has been stopped. Different variant of rotavirus vaccine are under developing on the basis of major rotaviruse anti genes able to form virus-like particles. Number of similar research shows efficiency of vaccine application at the model system [19, 32]. Technology of recombinant DNA founds wide use in investigation of rotavirus vaccine. There were created and are under studying living vaccines on the basis of smallpox vaccine and living bacterial vectors (E.coli, Salmonella, Schigella, Lactococcus) [18, 36]. Prospective results have been revealed on mice at application of recombinant vaccinia virus expressing VP7 rotavirus protein conjugated with influenza virus hemagglutinin transmembrane domain [10].

DNA vaccines are also under investigation. But obtained results are ambiguous in respects of its adaptability [16,17]. For recent years scientist's emphasis are concentrated at plants as a source for rotavirus recombinant proteins [25]. Technology on virus-like rotavirus particles production in tobacco-plant has been investigated [30]. At the same time, the plant expressing rotavirus antigens (proteins) itself can be used as a food substance intended for oral immunization. A multicomponent vaccine has been created comprising genes of rotavirus enterotoxin, subunits B and A2 of cholera's toxin and fibrillate antigene enterotoxigene of the E.coli, introduced into potato plant [35]. Tests on mice showed prospective results. It was reported about production of potato plants expressing the major capsid protein VP6 of bovine group A rotavirus that intended for application in diagnostics purposes [28]. In current research it is planned to perform plant's transformation with several genes (so-called multi gene transformation), which is seems to be most perspective since it allows to make several positive signs at once [20]. We suggest two variants for increasing plant's biomass. The first is cloning of vegetative sets by apical meristem cloning. The second is propagating by seeds. MM wave exposure is planned both for the first and for the second testing. Besides, it is planned to choose optimal regimes on increase of general biomass of plants.

The target of the Project

To create technology for application of electromagnetic generators radiating non-thermal mm waves to increase recombinant protein in plants and biomass of genetically modified plants.

Purposes:

1. To create plants producing rotavirus protein, which is the major factor of diarrhea for children and young animals.
2. To verify application of mm wave technique for biomass increase of genetically modified plants and recombinant protein production at model for rotavirus protein.
3. To study opportunity of mm wave application for optimized technology of genetically modified cloned plants at the pattern of transgenic plant, modified by rotavirus proteins.
4. To investigate opportunity of getting mucositis vaccine for prophylactic of rotavirus diarrhea for calves on the basis of recombinant proteins, producing by modified plant.

Expected results.

According to results of planned research on MM wave technique application for growth of modified plants the following steps can be taken: Opportunity of MM wave application in plant genetic engineering (survival of recombinant clones of plant cells), Optimal parameters for increase of recombinant protein



production, MM wave effectiveness on preplanting processing of transgenic seeds and general plant's biomass. The target of the investigation is formation of plant able to produce major rotavirus protective antigen producing both antibody and cell immune reaction based on technology of recombinant DNA with genes G/P dominant at Eurasia variants of human rotavirus and calf rotavirus. It is supposed to study effectiveness of rotavirus protein production in different vegetation periods, its content in various organs of the plant. In experiment on laboratory animals it is expected to find the effective immune response via dose, operating mode. It is supposed to study safety for vaccine and to testify its protective aspect.

#### Cost efficiency

It is presumed that MM wave technology will allow to increase biomass growth of transgenic plants, which in future might be applied in Agriculture for achieving high harvests of various transgenic plants.

Application of new plant, as result of experiments, in form of an eatable vaccine, could bring down damage caused by virus in cattle breeding, and vaccine selling or selling of technology designed under study will allow to cover expenses and to realize profits. It is necessary to underline that the suggested method for vaccine producing is of low prime cost, and it makes it competitive. Investigations on creation of eatable rotavirus vaccine for cattle will allow later on to test the suggested method for producing rotavirus vaccine on genetically modified plants for humans. Many developed countries suffer from rotavirus infection. In USA economical damage of rotavirus infection amounts to 1,5 - 2,0 million dollars per year.

#### Users of technology

Consumers of the investigated technology - manufacturers of genetically modified plants for Agriculture who pressed towards increased crops and productivity of transgenic plants. Results of investigation on efficiency increase of transgenic plants by MM wave as well as its productivity increase will be demanded by biotechnology laboratories and can be applied as an extra method for increase of crop capacity in various industries, connected with genetically modified plants.

One more aspect of the study is creation of eatable cattle rotavirus vaccine. Wide spread of rotavirus infection and economical damage of the disease are main reasons for high market demand of the vaccine. Farms and stock-farms all over the world are among main consumers of the designed product.

For 20 years in Russia a study on serotype and genotype variability of human rotaviruses has been performed. There were revealed dominant genetic models for the most spread G/P types of rotavirus and parameters of its temporary redistribution [4]. Rotavirus antigen dominated on the territory of Russia appeared to be the same dominant as in Korea [31]. There were designed technologies for getting recombinant DNA [24, 3, 8] and getting transgenic plants producing virus proteins [1]. We have experience at immunity (humoral and cell) estimation [26, 27].

A Bank of genes has been created on the territory of Russia, comprising structural and non-structural rotavirus protein for human and cattle [13]. Three patents on recombinant plasmid DNA containing protective antigen of human rotavirus dominant at Euroasia territory were granted [5,6,7]. Bank of rotavirus genes makes possible to start work on creation of transgenic plants and start investigation on MM wave application for the purposes of plant genetic engineering and its influence to biosynthesis of cloned proteins in modified plants and general output of biomass for genetically modified plants.

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## Fish



Background of the problem: Method for increasing viability of bioobjects

The developed method allows to provide:

- increasing fish livestock of valuable breeds fishfarmed in closed waters;
- growth of algae's biomass, grown for obtaining food supplements and pharmacological preparations;
- improvement of general ecological situation in closed waters, meant for farming of fish and other inhabitants of aqueous medium; in particular, prevention of algae decay;
- decrease of expenses on antivirus and other preparations meant for the enhancement of closed waters .

The essence of this method is that influence is performed by means of MM -range low intensity nonionizing electromagnetic radiation source located in aqueous medium.

The choice of quite narrow EMR wave length range for receiving effect on increasing viability of plants and animals – inhabitants of closed waters – is based on theoretical and experimental research, proving that MM-range EMR performs normalizing effect on metabolic processes which proceed in living cells. MM-range EMR influence on plants and/or animals stimulates synthesis in cells of biologically active substance ATPH (adenosine triphosphoric acid), which serves as a source of chemical energy in cells.

Locating of MM-range EMR-source in closed water allows to put all the inhabitants of the pond under the mentioned normalizing influence: plants, animals, microorganisms, including algae and fungi. As a result the viability of the closed water inhabitants increases remarkably.

The second essential feature of this method is that aqueous medium is used as EMR carrier. It is known that MM-range electromagnetic waves are absorbed in water most extensively. Nevertheless, it was stated experimentally that EMR underdamping effect is observed in aqueous medium within  $60 \pm 10$  GHz frequency band. Optimal parameters of irradiation, which provide practically loss-free EMR spreading in

aqueous medium, are defined depending on particular physicochemical parameters of aqueous medium (pH, salt composition, temperature, etc.). Consequently, it is possible to expose the inhabitants of significant in area pond to MM-range EMR-influence using this method. The water, which have been put under MM-range EMR, develops distinct ability to preserve the acquired properties for a long time. This allows to perform water irradiation periodically. It was also revealed that it is not necessary to use high capacity EMR generators to achieve the viability increase.

MM-range generators with capacity for 0,5 to 1,0 mWatt were used for the method execution.

For algae MM-range EMR influence was performed in an early stage of algae development. MM -range EMR-source with capacity for 1,0 mWatt was immersed into the pond daily during 10 days. Each irradiation period lasted 30 min. With *Spirulina Platensis*, which had been exposed to MM -range EMR, algae biomass output increased by 92% in comparison to unirradiated algae biomass output grown in control ponds, all other conditions being equal.

In experiments for determining optimal mode of MM -range EMR effect on young fish with the purpose of its viability increase the chosen ponds were inhabited with mirror carp and with special species of sturgeon fish. The experiments resulted in fish livestock growth by 112% in the irradiated by MM -range EMR ponds in comparison to the control ponds, all other conditions for fish keeping being equal. On conducting the experiments optimal modes of EMR influence were determined (irradiation source power, periodicity property and influence duration) depending on surface area of the pond, quantity of fish in it, age of the fish, ambient temperature, etc.).

Thus, this method for increasing viability of bioobjects can be used successfully in agriculture for artificial growing of different flora and fauna species.

## Yeast

Materials and methods.

The following microorganisms were taken as objects of study:

- Mycelial fungus: *Aspergillus orizae*, *Aspergillus awamory*;
- Yeast-like fungus: *Endomyces fibuliger*;
- Yeast: *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*.

A wide spectrum of characteristics for each organism was studied, but special emphasis was put on properties typical for each organism.

Biological methods of MM wave influence on microorganisms were under investigation at special biology institutions (biophysical faculty of Moscow State University, Scientific and Production Association of beer and soft-drinks, Russian Scientific Research Institute of fermentative products, Russian Scientific Research Institute of antibiotics and ferments for medical purposes).

Study of MM wave influence on Yeast-like fungus.

Two types of yeast-like fungus were taken into consideration; *Aspergillus orizae* (culture MSU) – amylolytic and proteolytic ferment producer, used for saccharification preparation, especially at incomplete starch saccharification. Proteolytic ferments of the fungus possess fibrinolytic properties, and thrombus dissolution is an important property of the fungus.

MM wave exposure stimulates biomass increase for *Aspergillus orizae*. Maximal biomass increase at exposure on fixed wavelength amounts to 22%; it is possible to decrease biomass output by 11% providing exposure at another wavelength [6].

Optimal operating mode for mm wave exposure stimulates fermentative activity for fungus, and stimulating effect is more evident for weaker initial material (no matter by natural or artificial factors it was caused – storage, temperature conditions, etc.).

It is possible to direct fermentation processes for *Aspergillus orizae* by MM wave exposure: to increase one process with simultaneous decreasing of some other process. So, increasing fibrinolytic activity by 80-90%, MM wave exposure could at the same time decrease caseinolytic activity up to the certain value. It should be marked that in contrast to bacteria, only multiple exposures by MM wave could influence on mycelial fungus spores. Repetition factor is 10 times. Changes acquired in the result of multiple exposures by MM waves are inherited by the next generations.

*Aspergillus awamory* 466 – is known as an amylolytic complex ferments producer. It has high glucoamylase activity and it practically doesn't produce proteolytic ferments. It is applied in biotechnology, in particular in alcohol industry, where starch to glucose hydrolyzation is required by glucoamylase and alpha-amylase ferments.

The same features as for fungus *Aspergillus orizae* [ ] has been found upon MM wave impact on spores of fungus *Aspergillus awamory* 466. An opportunity to control fermentative activity was revealed.

Maximum increase of alpha-amylase activity (up to 67%) is obtained at decrease of glucoamylase activity by 30%; selecting exposure mode, it is possible to get repression for alpha-amylase synthesis.

Stimulation of *Aspergillus awamory* 466 alpha-amylase activity leads to increase in starch hydrolyzation in biotechnology operations, for example, in alcohol technology. MM wave impact gives opportunity to restore fermentative activity of the fungus, reduced due to natural or artificial factor influence, up to the initial values. For getting stable effect by MM waves the multiple exposures to spores of fungus are required. Repetition factor is 10 times. Changes acquired in the result of multiple MM wave exposures are inherited by further generations.

Study of possibility to control yeast-like fungus *Endomyces fibuliger* by MM wave exposure.

*Endomyces fibuliger* is a yeast-like fungus, characterized by well-developed true mycelium, which easily come apart on separate cells; no gemmation. It is an amylolytic ferments producer. Under exposure of MM waves some morphological changes are marked – for example, priority of yeast cells was observed, while for non exposed culture mycelium typhus dominates; stimulation of biosynthesis for amylolytic ferments: increase of glucoamylase activity from 35 up to 49% was observed; increase of alpha-amylase activity ranged from 43 to 66%. These data were obtained at selection of MM wave exposure parameters and culture mediums [7]. MM wave exposure also could directly decrease fermentative activity for fungus.

MM wave exposure improves fungus fermentative activity during storage. Obtained during multiple MM wave exposures properties (10 repetitions) last for a long period and are inherited by generations [8]. MM wave impact on microphil fungus *Dacthilyum dendraides* revealed possibility to regulate protease activity for *Dacthilyum dendraides*, in particular protease activity increase by 50%, adjustment of fermentative activity shift both to direction of fibrinolysis process and to caseinolysis process (up to 42%). A special interest to the fungus mentioned owing to the fact that along with protease which could dissolve fibrin in vitro, it could form fungicidal antibiotic against phyto-pathogenic fungus of agriculture vegetables. It seems possible by means of MM waves effect to increase output of antibiotic which is used in agriculture for pest control (phyto pathogen fungus).

Study of possibility to control yeast cultures by MM wave exposure.

Study of MM wave exposure to yeast culture had been observed on two strains: Alcohol yeast - *Saccharomyces cervisiae* – is applied at alcohol production by biochemical method, with grounds on the yeast vital activity, the said kind of yeast turns glucose from the nutrient medium to alcohol.

MM wave exposure at certain wavelength allowed to speed up biomass accumulation by 53% at yeast growing in aerobic conditions [4]; initiated changes in cell cytology due to cell length changing - cell length increased by 40-50%, length to width ratio multiplied by 1.5 times. MM wave exposure stimulated functional biosynthetic yeast activity, which was proved by changing glucose utilization character by yeast: in particular maltose fermentation increase by 75%, glycogen cell content by 35-40%, which in his turn, increased fermentation processes, that means shortening for biotechnology cycle.

Changing in yeast fermentation activity, acceleration for biomass uptake and other properties resulted from single MM wave exposure is repeated in the next generations. Using method designed by N.B. Bychkova, monitoring for growth speed of yeast race upon MM wave exposure had been provided.

Biological effect for growth speed changing was observed for 300 cell generations. [8,°].

So, MM wave exposure on yeast culture *Streptomyces* spheroids gives possibility to change cultural, cytological, physiological properties for culture and save the obtained changing for many generations. Brewer's yeast - *Saccharomyces carlsbergensis*. This kind of yeast is used in brewing production at two technological stages: stage of major fermentation, which is characterized by intensive fermentation of wort carbons and yeast sedimentation upon fermentation finishing; stage of after-fermentation characterized by intensive fermentation of the left glucose, beer ageing which main criteria is volatile compounds, spoiling taste and odor for beer, such as diacetyl and aldehyde.

Study on MM wave influence on brewing yeast had been conducted for several years. A great number of properties were taken into consideration. Finally, a method for speeded process for brewing production has been worked out and laboratory equipment for testing the method, laboratory tests and tests at the Moscow experimental brewing plant [11]. It was shown that MM wave application (changing parameters and modes) gives the following results:

1. Changing curve for growth character. Lag phase is 2.3 times shorten (phase of culture adaptation to the medium), stationary phase is 6 times shortened, that means changing in growth speed for yeast culture. These changings are memorised by cells and inherited by further generations.
2. Fermentation activity increase at 19 – 31% and flocculative property increase at 20 – 50%. The index for flocculative property increase depends from the same value for initial culture. The lower initial index for non exposed culture, the greater index for exposed culture, no matter which reasons - natural or induces – caused its decrease. Series of results for brewing culture, which vital activity had been artificially suppressed by chemical agent (Surface Active Agent) cell processing have revealed that MM wave exposure not only renew artificially reduced vital activity, but recession mostly protected (see table 3,4).
3. Maltose fermentation increase in 1,2 times, glucose and fructose in 1,1 time.
4. Decrease of diacetyl and aldehyde number at 20%, which leads to after-fermentation speeding, that allows to short brewing technological process.
5. Decrease of vitamins necessity for cells (thiamine, inositol)

Table 3.

Influence of MM wave exposure on biomass synthesis for *Saccharomyces carlsbergensis* race II under conditions of graduated repression.

Reagent (repressor)	Reagent concentration, %	Unexposed culture		Culture, exposed by MM waves		% reduction
		Biomass, gram/liter	% to the control	Biomass, gram/liter	% to the control	
Saccharose	0	16				
	5	.2	32.5	25.6	15	6.2
	25	4.1			5.8	41.3
Natrium lauril-sulphate	0	15.9	12	1		
	0.025	10.7	76	1	67.3	
	0.05					17.4
Twin 20	0	16.6	8.6			
	0.5			51.8		
					12.6	10.0
						79.4
						62.8

6. Properties acquired in result of single MM wave exposure remaine for further generations. As an example, behavior repetition for brewing yeast *Saccharomyces carlsbergensis* was observed for some industrial generations. Yeast intended for wort is used many times (seed-yeast) in brewing industry. Upon fermentation finishing, yeast sunken to the button of the fermenting tanks is taken off and used for the next fermentation seeding. The described cycle is called industrial generation, it could include more than 100 cycles for cell reproduction. Results for behavior testing resulted from MM wave exposure are listed in Table5.

Table 4.  
Influence of MM wave exposure to the vital recession rate for *Saccharomyces carlsbergensis*.

Reagent (repressor)	Reagent concentration, %	Content for dead cells		% of renewing
		Unexposed culture	Culture exposed by MM waves	
Saccharose	0			
	5	8.0	15.1	25.3
	25			9.3
Natrium lauril-sulphate	0	8.0	16.4	74.3
	0.025			10.4
	0.05			12.5
Twin 20	0	7.5	13.4	13.3
	0.5			10.0
	2.0			10.5
				10.8
				92.8
				83.3

The listed results certify that multiple applications of initially exposed yeast by MM waves doesn't influence to resulted properties, the cell behavior is repeated for many cell generations.

Industrial testing confirmed opportunity to shorted times for industrial brewing. In laboratory conditions saving time is 1.5 – 2 days. In industrial conditions it is one day. So, talking about general results of MM wave exposure on yeast it is possible to point out the following regularities:

1. MM wave exposure influence at all vitality features for microorganisms: morphological, cultural, physiological, biochemical.
2. Generally, it is possible to find parameters and modes of exposure, which application allows to increase growth speed for cells and biomass increase.
3. Changing MM wave exposure parameters it is possible to control ferment's biosynthesis for microorganisms, which in its turn, gives opportunity to get new forms of microorganisms with directed ferment synthesis.
4. The effect of MM wave exposure the most evident for the cases where initial cell vitality had been suppressed by artificial or natural reasons compared to the initial vital activity.



5. MM waves revealed protective effect. Preliminary exposure by MM waves allowed to a greater extent to protect repression.

6. Behavior resulted by MM wave exposure lasted out for a long time and inherited by the next generations.

Table 5.

Influence of MM wave exposure to behavior of *Saccharomyces carlsbergensis*.

Index	1 industrial generation		3 industrial generation		6 industrial generation	
	Unexposed	Exposed	Unexposed	Exposed	Unexposed	Exposed
fermentation activity per hour, ml	53.3	62.6	51.2	60.3	41.3 45.6	53.6
Duration of fermentation, hour	106	84	108	89	102	85
Flocculate ability, cm/40 micron	1.2	1.4	1.2	1.4	1.2	1.5
Number of glycogen-holding cells, %	25.5	33.1	29.2	38.8	32.8	44.2
Diacetyl content, ml/l	0.33	0.28	0.27	0.24	0.28	0.28
Aldehyde content, mg/l	19.0	16.0	28.7	26.0	19.5	17.9

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## Poultry



State of the questions based on literary and our own data

Numerous researches data concerning low-intensity mm-range electromagnetic radiation impact on living organisms (BEMZ, 1999; Shironosov, 2000; Rakovic, 1999; Dennett, 1991) allow supposing that it is possible to achieve favorable influence on the course of treatment for many diseases.

In Russia Alexeev and Ziskin (1999), Rojavin and Ziskin (1998), Devyatkov and Betskii (1994) considering their research results established that mm-radiation influence amplifies and speeds up organism's fight against the disease (defense potential) by mobilizing its own abilities inasmuch as age and different factors, which upset vital activity normalization, allow.

Number of researchers (Dikke, 2000; Dedick, Korolyov, 1999; Balibalova, Korolyov, Rebrova et al. 1995; Georgiyev, 1983; Frohlich, 1983) registered favorable mm-electromagnetic radiation effect when treating mastitis and endometritis of cows.

Nevertheless there were no reliable research data available, which could prove electromagnetic radiation application in poultry industry, in particular, in meat poultry farming. Therefore, in 1996 preliminary researches on mm-range electromagnetic radiation influence on one day broiler-chicken viability were held in JSC «Lindovskaya» industrial farm, Nizhny Novgorod Region. The research authors – Glukhovskiy G.I., Krevskiy M.A. and Glukhovskiy N.A. showed that the percentage of feeble chickens hatched out of the eggs irradiated before placing into the incubator (experimental group), was as much as 2 times lower than the same index of unirradiated eggs (control group). The percentage of healthy chickens in experimental group was 82.3 against 77.7 in the control one. The preliminary researches also illustrated that average daily live weight increase of four day chickens irradiated by electromagnetic generators exceeded by 9% chicken live weight increase in the control group. Nevertheless reliability of discrepancy in indices was not proved.

### Urgency of the problem

In meat poultry industry the questions of dead chickens normalization, forage effective use and broiler-chicken productivity increase have not been solved yet.

Preliminary researches on mm-radiation application in «Lindovskaya» industrial farm confirm the obviousness and necessity to conduct further researches for establishing reliability of electromagnetic waves influence on growth, development and broiler meat productivity.

It is obvious that disease treatment and animal or poultry productivity increase is considered to be economically effective. The urgency of the problem related to the economic efficiency increase of agriculture production with new biotechnologies application is also obviously sustainable, however, this obviousness requires additional proofs introduction. In this context it is no doubt that it is necessary to conduct large-scale researches on the quality of agricultural production and quantity manufacturing, which indices increase with radiation application on animals and poultry.

### Purpose of the project

Development of the technology for application of Millimeter Wave Radiation generators to improve the productivity of meat poultry industry.

### Project objectives:

1. Establish mm-generators practical application opportunities for broiler poultry industry meat productivity increase in battery farms.
2. Determine optimum mm-radiation parameters (frequency, intensity, exposure, etc.) for broiler live weight increase while growing to the age of 60 days.
3. Identify the following qualitative data of broiler meat production after the use of MM-electromagnetic radiation in poultry farms:
  - o Controlling bacterial resistance, antibody kinetics;
  - o Growth, body composition, skeletal integrity and other biological traits;
  - o Physiological genetics; gene expression;
  - o Diseases and sanitation waste;
  - o Poultry products such as mechanisms and prevention of lipid oxidation in meat and poultry;
  - o Effect of irradiation on meat quality, utilization of poultry meat and egg components;
  - o Economic efficiency.

#### Expected Outcomes and Economic Effect

According to Russian and Western studies it seemed obviously that the application of Millimeter Wave Radiation (MMWR) therapy is itself to be a remarkable for animals' treatment and quite economically efficient. As a result of our own study in poultry the use of electromagnetic impulses will increase chickens' sanitation, decrease their diseases and improve meat productivity and broiler meat production at all.

Our hypothesis follows up to the use of generators and other equipment of Millimeter Wave Radiation, it will have positive effect on significant impact in meat production and also influence outcomes in meat quality fitting market standards of packed chicken meat. As for economic effectiveness we expect a breaking down the cost of meat production, we broadcast the impact of microwave usage on increase of poultry profitability and net production income.

#### Perceived population of new technology users

A number of milestones in the broad use of MMWR illustrate the efficacy of new technology and point to the prospect of future success of using this technology in Agriculture. The conducted research and the following studies is not to be a complete survey of the field but merely an illustration of real opportunities for wide users of MMWR generators and other equipment.

MMWR may have a profound effect on animals as well and there is an argument of the importance and implications of such work are new possibilities such as improvement of growth and development of animals and increase of their productivity. So that there are many users of new technology in agriculture such as beef and milk industries, poultry and swine production, corn, soybeans and processing industries and etc.

As for the geographical arena this technology does not have limitations to use, other words this technology is applicable not only in Russia and the U.S.A, but in any other countries worldwide.

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## Veterinary



### MM-therapy in veterinary

In the history of appearance, development and formation of mm -therapy there was a long-term research of mm-wave low-intensity electromagnetic radiation influence on living organisms (microwave range). The experiments were carried out on different animals. Numerous researches data allow to suppose that it is possible to achieve promising results on the course of treatment for many diseases, which this species of organisms can cope with, by choosing operating frequency (frequency range). MM -wave influence amplifies and speeds up organism's fight against the disease by mobilizing its own abilities inasmuch as age and different factors, which upset vital activity normalization, allow.

It is proved that signals like MM-wave are generated and used by organism itself for definite purposes and external irradiation only imitates these signals.

Penetrating into the organism these signals on certain (resonant) frequencies are transformed into informational ones, which maintain and control reconstruction or adjustment processes in it.

There are positive results of mm-therapy application in agriculture while treating a number of animal diseases, especially those, which are connected with inflammatory processes and immune system depression.

Mastitis – breast inflammation – nowadays is a widely spread disease; especially cattle are mastitis -prone. About 25% of cow livestock is ill with mastitis every year. The udder inflammation causes: machine milking rules violation, unsatisfactory keeping and feeding conditions. As a rule, cows with lowered immunity are mastitis-prone. Standard methods of treatment are based on application of medicines: antibiotics, sulfacilamides, nitrofurans and their combinations. Such animals give milk, which is harmful for people and unfit for foodstuff production.

Endometritis – uterus mucous membrane inflammation – quite frequent post-natal cow disease; medicines are also used in such case for treatment and milk from such cows cannot be used for food till they recover.

Evidently, treating of the mentioned diseases without medicines or by using less of them and treating period shortening are considered to be economically important. These ideas stimulated working up of corresponding equipment and mastitis and endometritis treating techniques, which imply mm -therapy.

Technique elaboration and checking gave the following results. When treating mastitis simple forms by mm-therapy after 10 séances 70 % of animals showed positive results, condition of 30 % of animals improved and treating was prolonged. When treating mastitis forms complicated by suppurative infection

mm-therapy was accompanied by antimastitis preparation single dosing; after 10 séances recovered 60 % of animals, the rest needed prolongation of the treatment.

Endometritis treatment by mono MM therapy was carried out during 10 séances. During approbation and testing 80-90 % of animals showed positive result. MM-therapy application was also effective for endometritis prophylaxis. Treatment started immediately after placenta separation; 5 séances were enough for none of the animals to fall ill.

There are positive results of mm-therapy application in different veterinary fields:  
Horse breeding (When treating myositis, arthritis, enterocolitis, emphysema and overstrain connected with excess physical exertion).

Horned cattle.  
Veterinary practice on pets.

Mass keenness on pets – cats, dogs, very often quite expensive breeds – also attracted veterinaries' attention to mm-therapy.

MM-therapy is a new technique for veterinary practice and it differs from existing physiotherapy procedures fundamentally and favorably. In certain cases it replaces drug and even surgical treatment techniques.

Besides, MM-therapy is highly remunerative. Thus, approximate apparatus price is 100 000 roubles, but considering the fact that modern drugs cost much it will pay for itself after curing several tens of animals.

It is extremely important that many drugs have cumulative properties and store in animal tissues and organs. MM-therapy technique allows to get ecologically clean animal foodstuff: milk, meat.

Conclusion. Thus, microwaves are economically profitable therapeutic means for veterinary purposes and for ecologically clean foodstuff production increase.

## APPENDIX

Horse breeding.

There are case records given below. The treatment was performed in industrial infirmary conditions of horse & sport complex "Bits" and MIA (Ministry of Internal Affairs, Moscow) cavalry battalion.

2-year-old stallion "Guanit". The diagnosis was hoof joint chronic arthritis of left pectoral limb. The horse was lame during one year, in spite of the fact that intraarticular injections of corticosteroids and antibiotic were applied and also phenyl- butazolidon preparations line was given intravenously.

After 10 séances' in the withers area limping passed.

5-year-old stallion "Phenomen". The diagnosis was enterocolitis, accompanied by constant diarrhea with liquid feces discharge and periodical colic pains. Symptomatic and pathogenetic horse treatment was carried out during 1,5 months but positive results were not achieved. Further mm-therapy was applied daily during 10 days. After 10 séances diarrhea was over, feces was formed, colic pains were not observed. The stallion was discharged from the infirmary clinically healthy.

6-year-old stallion "Zhezi". The diagnosis was radiocarpal articulation arthritis. Intermittent left pectoral limb lameness was observed during 6-7 months. Corticosteroids introduction (2 ml of dexasone with 2 - days' interval in the joint cavity) was ineffective. After 10 mm-therapy séances the horse stopped limping and since October 1991 recurrence was not observed.

5-year-old stallion "Buzuluk" was taken to the infirmary with lungs alveolar emphysema diagnosis. The horse had cough, rales, hard tense respiration, rapid pulse, excess fatigue, it refused to work. After mm-therapy treatment (10 séances with 30 minutes' treatment) characteristic for lungs emphysema symptoms

passed, the horse recovered his appetite, general tonus increased, it became possible to use this stallion for work.

The following stallions: 7-year-old "Zemlyak", 6-year-old "Kovyl", 6-year-old "Diagnoz", 5-year-old "Zaman", 5-year-old "Bazar", 5-year-old "Bedoviy", 4-year-old "Volkhov" and 12-year-old "Hamburg" after participating in film-shooting in the group of stunt men were taken to the infirmary with the same diagnosis: overfatigue, overstrain, weariness, some of the horses lost a ppetite and fatness. The animals were treated only with mm-radiation (7-10 séances). As treatment result the horses recovered their appetite, general tonus, they began to move actively.

8-year-old stallion "Ingas" had lumbar area acute myositis with the s ymptom of severe pain. Corticosteroids intramuscular injection, blood autotransfusion with 0,5 % novocain solution, massage during 1,5 months did not give positive results. After mm -therapy application (10 séances according to usual technique) the horse recovered and started training.

Horned cattle.

Mm-therapy was used on cattle for gastrointestinal diseases.

In Khrenovsky plant in the Voronezh Region 8 calves of Ayrshire breed at the age of 3 -4 months were treated against bronchopneumonia. The diagnosis w as set on the basis of clinical symptoms: cough, fever, general depressed state and sometimes refusal to eat. During lungs auscultation moist and dry rales were heard. Disease period lasted from 5 days to 10 days. 7 calves were in a moderate condition, one calf was in a very bad condition.

During first 3 days the treatment lasted for 40 min, later on - 30 min. Other therapy techniques were not applied. After 6 séances general state of all animals noticeably improved: cough stopped, short breath passed, the temperature normalized, appetite recovered. After 8 séances 7 calves clinically recovered.

In collective farm by M.Gorky in the Moscow Region EHF apparatus industrial testing was held on 7 calves with bronchopneumonia symptoms: fever, depressed state, bad appetite, tachypnoe, during auscultation moist rales were heard.

After 10 treatment séances 4 calves had normal temperature, recovered appetite, they became active, the rales passed. Other 3 calves after treatment course had an improved general clinical st ate, though rales and tachypnoe vesicular respiration were still observed.

In the cases with cows in the farm "Bessonovo" of educational farm "Leonovskoye" afterbirth retention was observed. Mm-therapy application was by contact method in 3d -4th lumbar vertebrae area during 30 min. In 2-2,5 hours after mm-radiation influence afterbirth separated from 5 cows at the age of 3 -6 years; from 2 cows afterbirth was separated manually.

In collective farm "Detskoselsky" in the Leningrad Region microwave electromagnetic radiation was used for afterbirth separating from 15 cows. Radiator was applied in 3d -4th lumbar vertebrae area during 30 min. After the treatment afterbirth separated only from 4 animals (26,6 %). It was determined that low mm-radiation therapeutic effect in afterbirth retention case is explained by placenta fused to the uterus.

Veterinary practice on pets.

On dogs mm-therapy was applied in the following pathologies: hurts, ligaments and muscle sprains, bones and joints deficiency, otitis, gastroenteritis.

Veterinary doctors V.S. Kuzin and S.V. Sereda in experimental self -supporting veterinary polyclinic "Center" (Moscow) in dog treating got the following results.

Male dog "Lois" of American cocker breed at the age of 1 year was ill with bilateral chronic otitis. Carried out drug treatment was ineffective.

During mm-therapy electromagnetic field was directed on the left ear tragus (left ear was chosen because it was more affected). Exposure lasted 30 min with one day interval; 10 séances were held. Auricle and

alveary cleaning with tight cottonwool tampon was performed every day, especially thoroughly immediately before treatment.

After 3-5 séances exudation quantity and painfulness noticeably lowered. After course of treatment was over the dog clinically recovered.

Male dog "Flint" of French bulldog breed at the age of 1,5 years had acute gastritis which was diagnosed on the base of clinical symptoms: lowered appetite, offensive odor from oral cavity, vomiting with bile admixtures, pain reaction during stomach palpation. 5 séances were held on alternate days with electromagnetic radiation direction athwart to spine in the area of 5 -7th thoracic vertebrae; treatment time was 30 min. After 5 th séance remarkable general state improvement and animal clinical recovery was observed.

6-year-old Alsatian female dog "Katy". The diagnosis was bilateral chronic otitis. The symptoms are: auricle redness, excessive exudates, painfulness. The dog was treated with drugs many times but without result. 8 mm-therapy séances were held on alternate days. Radiation was directed on the auricle tragus. Treatment time was 30 min. After 3 d séance the downturned left ear tip rose, pain reaction during palpation lowered remarkably, redness reduced. Full clinical recovery of the animal came after completing the treatment.

9-year-old Labrador male dog. The diagnosis was dermatitis in the lower jaw area. Earlier drug treatment was not applied. Electromagnetic wave radiator was applied to skin lesion area. Treatment time was 30 min. There were 4 séances held on alternate days in all. After completing the treatment tuberosity and thickening passed, painfulness was not observed.

According to "Biocenter" veterinary doctors A.F. Ashatkov and others' data mm -therapy dog treatment was held in animal owner's domestic conditions. The EHF apparatus radiator was put directly on the affected area. 10 days' treatment was performed daily during 30 min. Drug treatment was not applied to the animals.

6-year-old rottweiler "Devil". The diagnosis was sprain and rupture of ligaments and shoulder girdle muscle. Lameness was over on the 15 th day after the treatment started.

2,5-year-old black terrier "Don". The diagnosis was right Achilles' tendon sprain of ligaments, lameness during 2,5 months. Recovery came on the 10 th day after mm -therapy application.

2-year-old rottweiler "Dan", lame in the left hand. Lameness etiology was not determined. Limping passed on the 14 th day after the treatment started.

6-year-old German shepherd dog "Ricksha". The diagnosis was hurt, pelvic girdle and gluteus muscle sprain of ligaments. The dog did not rise during 10 days. On 3 -4 th day after mm-therapy course started the dog began to get up and on 9 th day it already made runs.

In Moscow Veterinary Academy clinic mm-therapy application in surgical pathology had the following results.

2-year-old dog of Irish terrier breed. The diagnosis was radial bone crack fracture and traumatic myositis in the affected area. Local treatment was made in pathologic nidus area. The exposure lasted 30 min. daily. After 5 séances limping remarkably reduced and after 10 séances lameness passed.

2-year-old cat got under observation with the left pelvic limb distal part contracture diagnosis and third - degree hurt in the femoral area.

Remarkable state improvement of the animal came after 10 days' daily treatments during 30 min, it started to move actively, the contracture reduced.

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## Medicine

By the beginning of the third millennium theory and practice of weak electromagnetic field interaction of millimeter range (microwave influence) with living organisms of different level of complexity are formed into a serious biomedical technology that has a very wide coverage of human activity scope from fundamental aspects up to practical and even residential usage.

Nevertheless, as every important technology this science in spite of its youth has a profound and interesting evolution history and is a part of international intellectual culture uniting creative contribution of eminent scientific of entire world.

In 1926 an outstanding Russian scientist Vernadsky V.I. set up a hypothesis that the biosphere and its fundamental component - living substances - appeared on the planet as united, inseparable and entire form [1]. With the advent of human being on the Earth, the biosphere starts to transform, the activity of these transformations increases with the growth of scientific knowledge. On the other hand, all living substances and human being as a part of biosphere are feeling a constant influence of environment different factors such as light, temperature, humidity, gravitational field, atmospheric pressure and so on. One of the most efficient factors of environment are electromagnetic fields of natural origin.

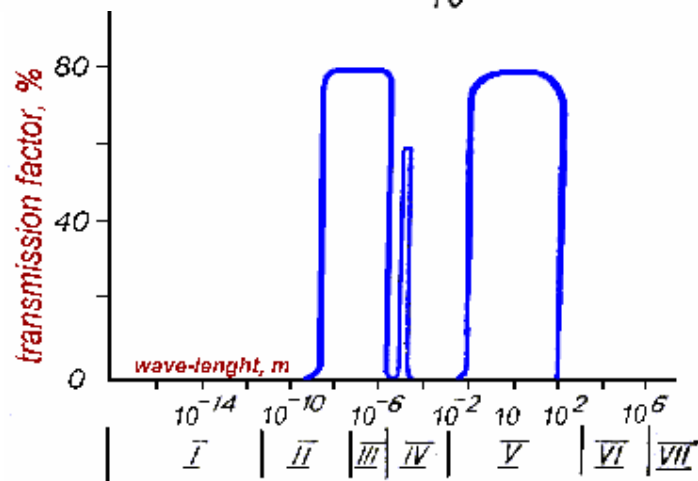
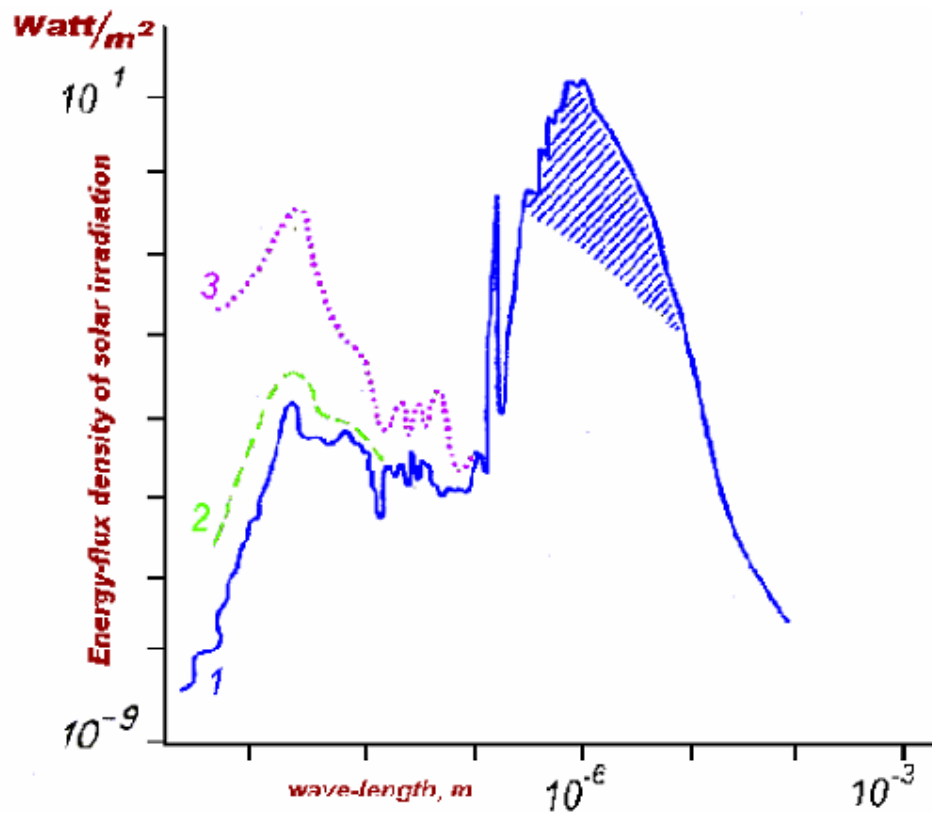
Vernadsky considered the biosphere as the Earth covers. The biosphere substance is penetrated by the energy. The source of this energy is located outside of the biosphere, in the space. Essentially, the biosphere is the part of the Earth's crust occupied by transformers converting space radiation into active earth energy such as electrical, mechanical, and thermal.

The range of natural solar radiation covers more than twenty regimes to the wave's length: from low-frequency radiation up UF, X-radiation and Y-radiation.

In spite of such a huge spectrum range of electromagnetic waves only a very little part of solar radiation and outer space gets to the atmosphere and reaches the ground surface. It is bound up with attenuation at the different atmosphere layers.

A maximum of radiation is located at the visual part of electromagnetic spectrum. This very radiation part in the range of wave-length (290-700) nm endows into the Earth energy supply and is characterized by constant (137+<sub>20</sub>) Wt/m<sup>2</sup>.

Shields allow electromagnetic radiation to get easily to its surface only in two spectral windows: optical ( $\lambda=290 - 700\text{nm}$ ) and radio-range ( $\lambda=1 \text{ mm} - 30\text{cm}$ ).



*Atmospheric windows  
for solar and cosmic radiation  
penetrating on the Earth.*

The absorption at the optical range is studied quite good and found its application in medicine (laser therapy, color therapy). As to the radio-wave range, the situation is more difficult. The follow-up researches have shown that absorption at the radio-range has a more fitful character. In particular, at the range  $\lambda=1\text{mm}-8\text{mm}$  a total absorption due to the oxygen on the all vertical atmosphere line could reach 800 DzB. [2]. The researches made by Kolbun N.D. illustrate that at  $\lambda=2.5, 1.7, 0.9, 0.8\text{ mm}$  bio-objects could react upon radiation exceeding a little a certain liminal spectral density capacity.

$$S = 10^{-21} - 10^{-19} \text{ W/GHz.}$$

This statement has a name of electromagnetic homoeopathy conception by analogy with a well-known medical homoeopathy, in other words, by using little drug doses essential changes are achieved in the

organism owing to the fact that these drugs were particularly selected.

By influence means such effects appear to be informative, i.e. they do not depend upon a powerful influence and they are characterized by influence parameters (information): range, depth and modulation level, polarization and other parameters.

Informational influences are transmitting through one of the transmission and information processing systems in the organism (acupuncture points system, nervous system, humoral system and possibly some other systems).

Upon such influence it is required that the power of actuating signal exceeds a certain threshold (minimal) level needed for actuating of transmitting system. The further signal increasing at the fixed limits dose not have any influence on system reaction.

Historically, first researches of informational intercellular interactions were carried out at the early 40th by the professor of Moscow University Gurvich A.G. He was dealing with one of the most difficult issues in biology: morphogenesis. He arrived at the idea that the mitosis - cells fission by two - could be only in case on combination of two independent factors: possibility factor - cell readiness to fission and realization factor - outer impulse. Gurvich unexpectedly suggested that possibility factor should be a certain type of outer radiation. The test has confirmed that suggestion!

At the present time this radiation is called Gurvich mitogenetic radiation. All animals and plants tissues, barm and bacteria radiate such radiation type.

On the 70th a German physicist Fritz Albert Popp paid attention on Gurvich researches. [5].

By using photosensitive multipliers, Popp has confirmed many results. From the point of modern quantum physics Popp showed that biological objects radiation of vegetative and animal origin differs of high coherence in the whole range from a close UF till a close RNA light. [6]. Usually, the coherence of bio - radiation is higher then the coherence of modern laser ray. To explain it, there was used quantum physics theory of American physicist R.Dikke.

In 1977-1988 G. Frolich substantiated theoretically and got experimental proof of the fact that all living cells generate alternating electromagnetic fields. He also evolved the general theory of coherent oscillations in the biological systems. According to Frelih's researches, [7], [8], biosystems have polarization (dipole) rippling at the frequency band of 100 -1000 GHz (3-0,3mm), cells vital functions processes transmit the energy to the local activated dipole rippling ("biological pumping"). The system can change into metastable state and from this state into the basic - "gigantic doublet" appears as a particular case of bio object coherent condition. The model assumes that such rippling cover macromolecule parts that reminds of low-temperature condensation of Bose condensate.

Thus, Gurvich predicted in the 20th of the last century the existence of intercellular in teraction not only at the optical, but also at the millimeter range. And only fifty years later academician Deviatkov N.D., theoretically substantiated and got experimental proof of the reality of such interactions.

Modern biophysics and modern medical instrument-making industry successes are connected mainly with the elaboration of one of the most productive and exciting physical theories of the day: with irreversible imbalance thermodynamics which describes self-organization and coherent structures appearance processes in chemical, biological and social systems. The author of this admirable theory is Director of Physics and Chemistry Department in Brussels University Iliya Prigozhin, the Nobel Prize laureate in 1977. [9].

Just as quantum mechanics originated from incorrectness in microworld objects describing by classical physics, as relativity theory arose on trying to describe object movements with rapidity near the velocity of light, so I. Prigozhin's imbalance thermodynamics emanated also from classical physics contradiction.

It is assumed in classical and quantum mechanics that fundamental laws of physics are symmetric in time. Since Newton physics considers its mission to achieve irrespective of time reality level on which mainly initial state evolves. A profound breakthrough in our minds made by relativity theory and quantum mechanics did not affect, actually, classical physics main principle. There is nothing in classical dynamics which could help to differentiate between the past and the future. Ther eby I.Prigozhin calls dynamics the physics of existing. Thermodynamics, in contrast to dynamics, he calls the physics of nascent.

The possibility to derive stable solution, far from balanced state, is an important result of new imbalance thermodynamics. This affirmation is a scientific basis of systems self-organization - it means initiation of coherent states on the base of random interaction of noncoherent structures. This principle is basic in physics. Developed by I.Prigozhin nonlinear imbalance thermodynamics theory, which describes processes in a living substance, is, in essence, fundamental physical basis of microwave therapy.

Living organism is principally imbalance a dissipative and coherent structure which has own frequency range. In particular, in the 60-ies academician Devyatkov N.D. with assistants revealed resonant character of interaction between radiation and bioobjects in the range between 30 -300GHz. It showed so called "informational" part of the radiation, in other words, possibility to manage biological processes on the level of intercellular interactions. [10]. Academician N.Devyatkov's great service was foundation of school for research workers oriented not only on concrete practical treatment results, but also on development of microwave therapy impact profound physical mechanisms.

Fundamental role of water and its different states are shown in the article [14, 15] for explanation of

microwave therapy impact. New registration method of microwave interaction with different objects is introduced. These researches also were held under Devyatkov's guiding.

#### REVIEW ON CLINICAL RESULTS OF MICROWAVE THERAPY APPLICATION

The results of experimental and theoretical research were the base of modern therapy methods - microwave therapy, which are widely used in clinical medicine. The big clinical experience of application of microwave therapy is described in publications of scientists and doctors, connected to the research group of academic Devyatkov ND [11-13].

In the publications by Devyatkov N.D. et al. [50-53] is analyzed the methodological particularities of microwave therapy in treating and rehabilitation of oncological patients, using rich clinical data (802 cases) the effectiveness of protecting, modifying, anti-cancer, anti-metastatic effects and results of rehabilitation and palliative therapy were researched. An experience of Kiev group concerning rehabilitation of oncological patients is presented in [21, 36].

A rich clinical and experimental data, demonstrated the changes in immune condition of the patients and experimental animals after low-power microwave radiation, provided due to changes in activity of immune cells [16-18] is collected. Immunostimulating effect of microwave therapy is extremely important for children, suffered with often inflammation diseases of respiratory system [19, 20]. The positive influence of microwave radiation on re-population properties of parent cells [21] was demonstrated, which is very important to solve the problem of improving the effectiveness of myelotherapy in hematological, immune diseases, in radiation disease, cancer and other pathological conditions, connected with disturbance of hemo- and immunopoietic function. It was demonstrated, that radiation of the blood of patients with gastric ulcer "in vitro" recover the decreased metabolic activity of leukocytes and phagocytic activity of neutrophils and monocytes [22].

Some authors reported a good effect of microwave therapy in treating children with chronic gastritis and duodenitis just as monotherapy [23], because the method has powerful immunostimulating, trophic and anti-inflammation effects. It was demonstrated, that microwave therapy decreases the absorption of iodine by thyroid tissue [24]. This is experimental base for application of microwave therapy in combined treatment of iodine intoxication as radioprotective method.

Circulatory diseases occupies the first place in invaliding and death causes in population. Insufficient effectiveness of existed methods of the treatment forces researchers to find new ways in decreasing of circulatory diseases. This new effective method is application of microwave therapy either as monotherapy or as a component of combined therapy. For example, application of official drug therapy in patients with 1 stage of arterial hypertension does not turn to normal the functional condition of circulatory system after the course of treatment, which can be detected by velocimetry, and in the case of 2 stage the common peripheral resistance remain relative high [25]. At the same time, the application of microwave therapy in the case of 1 stage of arterial hypertension can adequately recover compensation functions of circulatory system. In 2 stage of arterial hypertension microwave therapy provides good normalizing effect to circulation, decreases the general peripheral resistance, improves several general heart parameters. Reliable hypotensive effect was obtained in 80% cases of arterial hypertension [26]. The effectiveness of application of microwave therapy as monotherapy or as a component of combined therapy in coronary heart disease was proved [27]. In coronary heart disease microwave therapy decreases the count of incidences and helps to turn progressive phase to stable one, increases the adaptive resources and aerobic power of myocardium, which correlates with physical abilities of patients [28]. Using microwave therapy is discussed as affective method of treatment of coronary insufficiency in patients suffering with 1-3 stage of coronary heart disease, which may be in concurrence against official drug therapy.

Microwave therapy exercises a significant normalizing influence on the blood coagulation parameters in circulation diseases, specifically in coronary heart disease and myocardial ischemia [29]. Mainly, microwave therapy has good influence on anti-coagulation part of coagulation system (increasing of heparin level in the blood, increasing the activity of antithrombin-III as one of the most significant component of blood coagulation process).

Paper [53] describes three new clinical-physiological directions, which were born from traditional microwave therapy:

1) primary reception, connected with activity of inter-cell media (collagen) and intra-cell proteins (heart shock proteins);

2) effects and mechanisms of microwave influences on the body structures, related to vitality of the organism (reanimation areas and points);

3) effects and mechanisms of microwave influence on large joints, defined the high effectiveness in patients with specific condition of conscious, specifically with phantom pain and vegetative disorders.

It was proved the effectiveness of application of microwave therapy in case of stomach and duodenum ulcers [30-33], in neurological patients [34], in combined therapy of patients with uterus tumor [36], in erosion type gynecological diseases [36], in gynecology [37], in combined treatment of orthopedic patients [38], in urological diseases [39], in chronic non-obstructive bronchitis [40], bronchial asthma [41], in cerebral atherosclerosis [57], in prevention and treatment of paresis of gastro-intestine organs after

surgical intervention on large intestine [58], in treatment of children paralysis [42]. Anti -inflammation effect of microwave therapy is using in treatment of prostatitis and kidney diseases too [43].

For the treatment of dental diseases and different clinical forms of osteochondrosis double -frequency method of microwave treatment was applied [54]. Radiation was performed either in static or in dynamic mode. Modulation of microwave emission was performed using the frequencies of owner main biological rhythms of patients, or using frequencies used in low -frequency acupuncture. Comparative analysis of parodontosis treatment showed the high effectiveness of double -frequency microwave therapy. Treatment of osteochondrosis was effective too.

Methods of diagnostics of teeth, based on radiation by centimeter and millimeter waves, can be used instead routine X-ray examination. The examination in 0,04 -400 GHz frequency band showed, that due to lack of normal tissue into the tooth affected by caries, most effective detection of normal and affected teeth can be achieved in frequency near 35 GHz [55]. The research was performed using two wave -guides, the tooth was places between wave -guides and space between the tooth and wave-guide filled by silicon media. It was detected, that tooth pulpa absorbs the electro -magnetic influence, that may be used to elaborate new methods of treatment.

The tendency of development of modern research on biological and therapeutic effects of microwave therapy is looking and clinical testing of new curative frequencies, in relation to well -known - 42,53 and 61 GHz [56]. The presence of clear biological effects was detected in series of experiments with 2,5 mm wave length, also it's supposed therapeutic effect at 1,7; 0,9 and 0,77 mm. Bio -informativeness of millimeter waves in decreasing with increasing of frequency, because the level of noise is decreasing. The most natural bio-informative frequencies are ultraviolet, infrared and short part of millimeter band.

On the base of researches provided by academic Devyatkov's group a new biomedical direction - microwave therapy - was born. Now this direction is the part of ordinary physical therapy. Microwave therapy is a classic example of informative influence, where the treatment success is defined not by power, but some specific "information complex". One of the most important information parameter is the radiation frequency. Academic Devyatkov on the base of experimental data discovered the specific discrete frequencies, where the optimal biological effect of influence can be obtained. After collection of some clinical experience and discovering other biological -effective frequencies, future developing of device-constructing was in two ways: wide-range emitters of lower-power noise radiation and wide-range emitters with electronic or manual frequency changing and possibility to select the individual influence frequency. Nowadays the most advanced devices, which includes the possibilities of all of existing ones, is EHF device.

In the articles written by Nyzhny Novgorod group of microwave therapy (Borovkov, Zanozina, Runov et al) it was proved the decreasing of peroxide oxidation of lipids (POL), activation of anti -oxidant ferments [59] and it was detected, that just this influence assists to cell -protection and provides anti-inflammation, immune-stimulating, analgesic and neurotrophic effects.

Hereinafter some results of application of microwave therapy in treatment of the most serious complication of diabetes mellitus - diabetic polineuropathy - are presented.

At the moment of start the present scientific research on diabetes mellitus by microwave therapy there were exists all theoretical bases to suppose its effectiveness in diabetes mellitus. In fact, performed researches concerning the possibilities of microwave therapy for treatment of one of the most serious complication of diabetes mellitus - diabetic polineuropathy - had brought positive results.

Into the Neuroendocrinology Department of Regional hospital by NA Semashko 62 patients with diabetic distal polineuropathy (DDPNP) were examined and treated by microwave resonance therapy. Patients were divided in the following groups: A - diabetic type I patients and DDPNP under 5 years of disease; B - diabetic type II patients and DDPNP under 5 years of disease; A1 - diabetic type I patients and DDPNP more than 5 years of disease; B1 - diabetic type II patients and DDPNP more than 5 years of disease.

To define the range of normal parameters the control group with 26 healthy peoples in the age  $23,2 \pm 2,42$  years was examined. At the moment of examination those peoples have no any complains and in clinical, laboratory and instrumental tests there were no internal and neurological diseases detected.

Treatment of DDPNP was performed by influence on corporal acupuncture points of: ST36 (Zu -San-Li), ST37 (Shang-Jiu-Xiu), ST40 (Feng-Lung), SP6 (San-Yin-Jiao), SP8 (Di-Ji), K7 (Fu-Liu), LIV4 (Zhong-Feng), LI11 (Qiu-Chi), P6 (Nei-Guan), B60 (Kun-Lun) by microwave radiation using EHF device. During the course of treatment the selection of specific resonance frequencies, where the maximal absorption of microwave radiation was detected, was performing. During the examination some frequency and other parameters of resonance, which are specific for diabetes, were found.

The duration of the treatment course was 20-30 minutes. During the treatment patients were admitted by standard diet and base therapy (insulin and/or oral sugar -decreasing drugs).

For quantitative estimation of the clinical manifestations of DDPNP the rate scale of "Total Symptoms Scale" (Scott J, Huskisson EC, 1976) was used.

The quantitative evaluation of disorders in tactile perception in DDPNP cases was tested by "Neuropathy Star" (USA) device.

To define the presence and severity of DDPNP and to detect the effectiveness of provided treatment we used electromyograph system Neurocid-M CID 1541 MI (Havana, 1990).

The metabolic level was tested by glucose-oxidase method, glycolized hemoglobin was measured by column chromatography.

The level of total cholesterol, alpha- and beta-cholesterol, triglycerids into the blood of diabetic patients were measured by routine methods.

The research of the POL was performed by detection of induced hemoluminescence of the serum. Also the molecular products of POL (diene and triene conjugates, Schiff's bases) and anti-oxidant ferments (superoxididistutase, catalase) were tested.

To detect the deformity of erythrocytes (DE) the method of rigidometry was applied.

Obtained data were calculated by using routing statistics methods in "MEDST" computer software kit.

## RESULTS

In a short duration of diseases (up to 5 years) microwave resonance therapy reduces the irritative -pain syndrome both in DM I type and II type patients (up to 77,3 % and 68,0 % respectively). In long diseased groups microwave therapy reduces the severity of irritative -pain syndrome in DM I type group for 60,2 % and in DM II type group for 59,2 %.

Dynamic of the speed of transmitting the of impulse along the nerve (STI) is one of the important parameter of the effectiveness of administered treatment. It was found, that microwave therapy reliable increases STI both in diabetes I type patients and in II type (fig.5).

## STI increasing (m/sec)

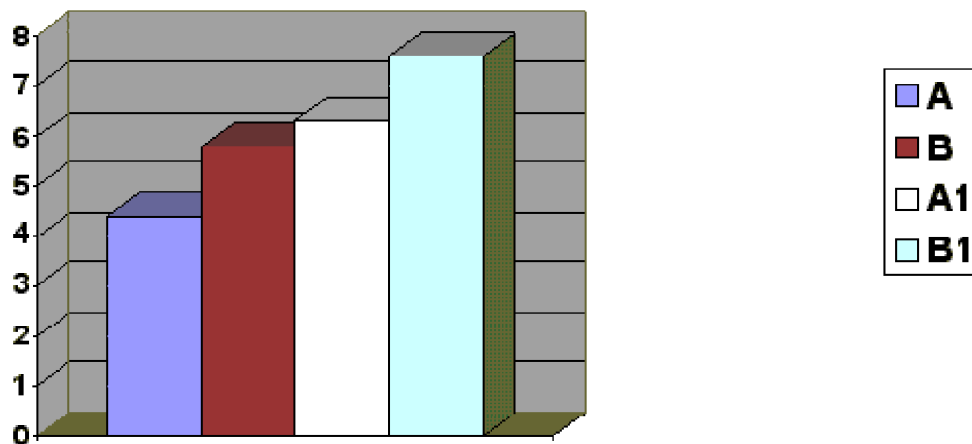


Fig.5. Average increasing of STI in diabetic patients with DDPNP induced by microwave therapy.

A - diabetes mellitus I type up to 5 years, B - diabetes mellitus II type up to 5 years,

A1 - diabetes mellitus I type more than 5 years, B1 - diabetes mellitus II type more than 5 years.

The research of POL processes showed, that in any duration of disease microwave therapy clear restricts the activity of POL, that manifests as regrassion of diene (DC) and triene (TC) conjugates (fig. 6 and 7).

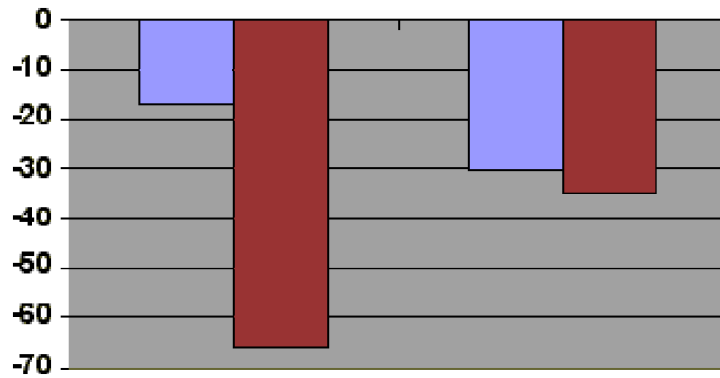


Fig.6. Dynamic of diene (DC) and triene (TC) conjugates in diabetes mellitus up to 5 years percent of decreasing of DC and TC.

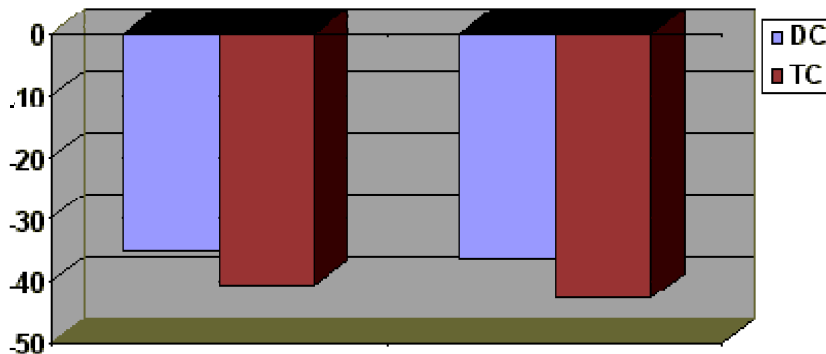


Fig.7. Dynamic of diene (DC) and triene (TC) conjugates in diabetes mellitus more than 5 years percent of decreasing of DC and TC.

Patients with DDPNP, even in short time of disease, have changed deformity of erythrocytes ( $34 \pm 4 \%$ ), that turns the endoneural hypoxia more strong. Microwave therapy improves this parameter for 28% ( $p < 0,01$ ).

#### CONCLUSION

1. Specific frequency and other parameters of millimeter resonance interactions in diabetes mellitus using EHF device were found.
2. Optimal parameters of influence in diabetes mellitus using EHF device were found.
3. Microwave therapy is effective method for treatment of some complications of diabetes mellitus, like microangiopathy (neuropathy, retinopathy) and polyneuropathy.
4. It was discovered the mechanism of microwave influence in diabetes mellitus, its base is in restriction of POL processes. It was demonstrated, that this mechanism is specific for microwave therapy.
5. It's necessary to perform additional researches on influence of microwave therapy on main components of pathogenesis of diabetes mellitus II type, i.e. resistance to insulin, disorders of production and secretion of insulin, which has a great practical importance.
6. One has technological possibility to produce high-effective low-cost devices for microwave therapy for using either by doctor or by patients with diabetes himself.
7. On the base of results of performed work specific type of microwave device for treatment of diabetes mellitus was constructed. This device operates in optimal frequency band.

Thus, on the base of performed radio-physical and clinical research we have a technology for production of different devices for microwave resonance therapy, working as fixed frequencies, as with possibility to change the frequency within 49-78 GHz band.

#### Resume

All the above mentioned demonstrates a clear conclusion. In Soviet Union and after its dividing in Russia the powerful science school of researches of microwave influence in different diseases was formed. The mechanisms of positive effects of microwave radiation were researched from the



fundamental base too.

Developing the medical application of microwave technologies before the experiments with cell cultures and animals is not usual standard way of developing of any medical technology in the World. The cause of this situation is great political and economic support just medical researches of microwave radiation in the period of Soviet Union. Above mentioned cause, as well as isolated position of USSR and, in many aspects, Russia from world market of medical technologies, different Russian standards of production of medical devices are the blocking factors for intervention into the world market of defined technologies of microwave treatment of many diseases. That's why now it's necessary to perform some relative little financial and managing efforts to make this technology as one of the world leading ones.

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First experimental research on interaction of millimeter wave with living objects had been performed on microorganisms in 1966-1978 by Vilenskaia R.L., Smolianskaya A.S., Gelvich E.A.[1 - 3]. At studying of mm-wave exposure at colicin synthesis it was found out threshold dependence for concentration for induction factor of colicin synthesis power flux density and wavelength. [4-6].

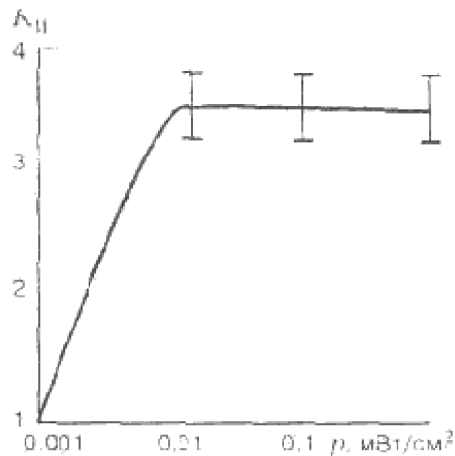


Fig.1. Inductance factor  $K_i$  for colicin synthesis via Power flux density of Electromagnetic Radiation. Sevastyanova L.A. was one of the first scientists who initiated study on biological effects of Low Intensity Electromagnetic Radiation of MM wave band on hemopoietic system of mammals in 1969 - 1971. She had obtained first positive results on reduce consequences of X-rayed marrow cells by preliminary MM wave action. Also it was performed evaluation of MM wave penetrating depth into animal's skin and was curtailed type of radiating power dependence for some animals and human. [10 -12]. Data being received at evaluation of penetration depth has shown that protective influence of MM wave radiation is of indirectly character.

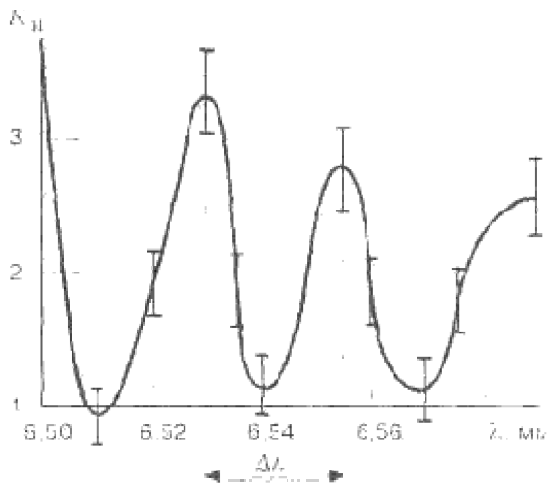


Fig.2. Inductance factor  $K_i$  for colicin synthesis via wavelength  $\lambda$ . During researches lasted for more than 12 years, there were tested over 12 000 experimental animals - mice and rats. Evaluation of hemopoietic system provided on quantity and status of marrow cells (rariocytes) of the left and the right femoral bone (as per the side of performed experiment). In some cases, calculations were made for Pancreas cells. The following Dependences discovered during researches are viewed below:

1. Biological Effect on Power flux density. At experimental series it was found out that one of characteristic properties of mm wave biological effects monitoring for both in

vivo and in vitro is a threshold dependence from power flux density, when after reaching threshold level further increasing of power doesn't effect the curve.

2. Biological effect on wavelength dependence. At combined influence "MM wave - X ray" at the following wave lengths 7,07; 7,10; 7,12; 7,15; 7,17; 7,20; 7,22; 7,25 and 7,27 mm the cell number of bone marrow cells of laboratory animals reached 85 - 90% if to compare with control number. (fig.3).

At influence of other wave length combination 7,08; 7,09; 7,11; 7,13; 7,14; 7,16; 7,18; 7,19; 7,21; 7,23; 7,24 and 7,26 mm, the number of bone marrow cells could be at the rate of X ray action only, that is 50 - 60% off the control value.

3. Biological effect of mm action in modulating mode. Due to strong dependence for biological effect of wavelength (which makes difficult to change proper wave length at clinic reality), a special device has been designed to ensure frequency modulating mode and number of experiments were performed not only at a unique wavelength but also at frequency modulating mode. Using such radiating mode if become possible to reach same effect as for wavelength with maximal effect. We came to the conclusion, that frequency - modulating mode could be applied at clinic.

4. Biological effect on mm wave action site. Nine experimental groups were taken into consideration: 1 - irradiation for right and left huckle; 2 - occiput; 3 - head; 4 - shoulder; 5 - animal side; 6 - abdomen; 7 - mixed radiation for all areas mentioned above but with mm wave only; 8 - X ray of left huckle only; 9 - control. At irradiation of occiput and huckle marrow cells affection was decreased at 7,11 and 7,13 mm wavelength. Similar dependence was revealed at irradiation of other animal sports (he ad, side, abdomen, should) but with application of different wavelengths. So, decreased number of affected marrow cells at mm wave localizations was resisted at 7,11 and 7,13 mm wavelengths and no effect observed at 7,10 and 7,12 mm wavelengths. (fig.4) These results prove that it is necessary to use unique wavelength for each of body area to reach biological effect.

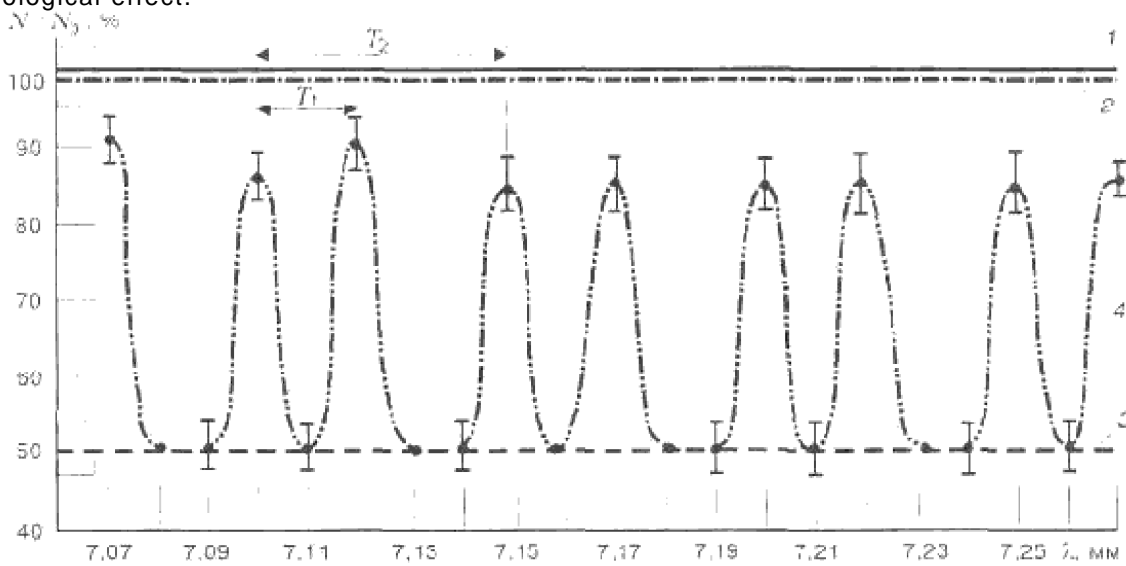


Fig.3. Marrow cell number via wavelength  $\lambda$  after complex MM wave and X ray action and after single X ray action :

1 - control

2- MM wave action

3 - X ray action

4 - complex action MM wave - X ray

T1 and T2 - period of resonance curve. Abscissa axis - wavelength (mm), ordinate axis - ratio of marrow cell number after action N to norm marrow cell number in norm N0 (%)

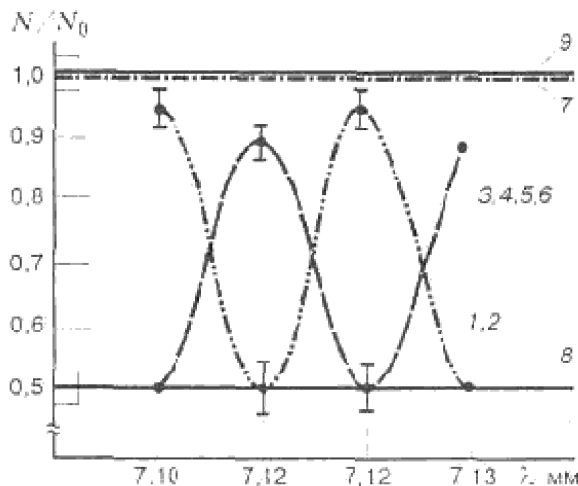


Fig.4. Changing in karyocyte number  $N/N_0$  (5) upon MM wave action to different skin area of animals in combination with local X ray exposure on hip. MM wave exposure (in combination with X ray) : 1 - to hip area; 2 - to occiput area; 3 - to head; 4 - to shoulder area; 5- to side area; 6 - to stomach area; 7 - exposure of mentioned areas by MM wave only; 8 - hip exposure by X ray only; 9 - control.

5. Biological effect on irradiated square. It was of scientific interest to check whether there is dependence of irradiated area or not and how sensitive is it.

General and local irradiation at mixed MM wave and X ray action gave similar biological effect. It was found out that even if irradiated area limited up to 10 mm<sup>2</sup> in mono frequency mode it is possible to get biological effect, but it is necessary to take care while placing radiating horn (even in several mm) since even small shift of horn (about several mm) requires change in wave length to 0,01mm, whereas operating in mode of MM wave radiation with modulation it is possible to get stable effect of decreasing involvement for marrow cells even at such small radiating spot on animal's body such as 10mm<sup>2</sup>.

So, supposition raised in 70th [22] about "frequency - amplitude windows" in electromagnetic spectrum where biological effects are of sufficient evidence, has got one of first experimental validations. It was found that biological effects of microwave radiation defined by its biotropic parameters (Intensity, Frequency, Signal shape, Localization, Exposition ets.) Study of animal's reactions at combined action of standard medicine, accepted in oncology treatment, and MM wave radiation disclosed the following: Response of animal's hemopoietic system to combined mm -wave and x-ray radiation.

It is known that according to x-ray radiating dose cells could obtain different infringements, expressed in chromosome aberrations, mitotic activity decrease and late cell mitosis, decrease of reproductive function. This in turn leads to decrease of marrow cells, degradation of blood cell elements.

Most of radio protectors, possessing low selectivity, with dose increase could produce toxic effects. Preliminary data gives evidence of protective properties of MM wave radiation and its selective action to marrowbone cells. More profound study has been made for mm wave action.

At combined action - mm wave radiation, x-ray radiation - it was observed less destruction for marrow bone cells than for x ray action only: until 5th day cell deficit amounted to 15%, while for combined action x-ray - mm wave cell deficit amounted to 38%.

1. Response of animal's hemopoietic system to combined mm -wave and anticancer agents.

Similar to x ray action anticancer agents initiate DNA dissociation, slow down DNA and RNA albumin synthesis. There are much similar in manifestations for X ray and chemo agents - chromosome aberrations, delay in mitosis stage and headway along cell phases, reproductive and interface destruction. Study of combined mm wave action and anticancer agents revealed that at certain parameters of mm wave radiation a destructive action of anticancer agents could be avoided, and functional activity of stem cells could be increased.

As for reactions of haematogenic system, its response, as increase to absolute number of karyocytes, appeared to be higher at all combined actions in comparison with x- rays

or anticancer agents only . Reduction of bone marrow cell damage was observed both for one-use and multiple-use of mm wave radiation combined with anticancer agents. Irradiation of animals with mm waves only didn't make changes in its hemopoietic system.

Malignant cells control for sensitivity to x-rays and anticancer agents is an important scientific problem. Application of practically all known agents or its combinations gives damaging effect to health tissues. Quite often toxic effect is so severe that it becomes necessary to limit course treatment. Such effect appears earlier than anticancer one.

Experimental results obtained by authors showed that MM waves didn't cause infringements for healthy cells. While, such type of electromagnetic radiation contributes regaining vital functions in tissues at destructions. For combined action of x rays or anticancer agents MM waves ensures protection. It happens due to increase of proliferative activity of stem cells in hemopoietic system, which, in turn, increases mitotic activity for bone marrow cells.

MM wave influence to animal's hemopoietic system at combined action with x rays and anticancer agent cyclophosphan had been studied during experiments. 1500 animals were tested. The following results have been received.

1. Influence of MM wave exposure on transplantable tumor and hemopoietic system for animals with malignant tumor. It was revealed that mm wave exposure neither slows down nor speeds up the growth of tumor.

An interesting fact could be marked for all experiments: all animals exposed by MM waves had life span on 10-15 days longer in comparison to control group.

2. Combined exposure of MM waves and X rays on transplantable tumor and hemopoietic system for animals with malignant tumor. Study of combined exposure of MM waves and X rays were performed on transplantable tumor RShM -5 on CBA line mice. Influence of these factors was effected in the following methods:

- o Tumor exposure with MM waves with further exposure of X ray;
- o Tumor exposure with X ray with further exposure of MM waves;

Along with these groups two control groups were taken into consideration: group with mice exposed with MM waves only and group with mice exposed with X rays only. For cases X-rays - MM waves it was observed a strong inhibition of tumor growth. , by 30th day it reached up to 80 - 90 %. In case of single X ray exposure or at combined exposure MM waves - X rays growth inhibition by the same period amounted up to 60 - 65%.

Reduction of bone marrow cell number practically had not been observed for case MM wave - X rays, by 7th day number of bone marrow cells was at the norm rate.

In case of exposure with X rays only cell restoration was slow and by 7th day the number of karyocytes hadn't reached control rate.

In case of exposure with X ray - MM wave number of karyocytes by 7th day reached physiologic norm.

Study of peripheral blood during exposure period showed that number of erythrocytes and leucocytes was higher for animals experienced by combined exposure of MM wave - X ray than for exposure on x ray only and X ray - MM wave.

3. Combined exposure of MM wave X ray on transplantable tumor sarcoma -45 and hemopoietic system of animals with malignant growth. There were tested the following experimental exposures:

- o MM wave exposure of tumor followed by X ray;
- o X ray exposure of tumor followed by MM wave;
- o MM exposure on thigh followed with X ray on tumor and again MM wave exposure but on tumor - that is double combination: MM wave - X ray - MM wave.

Strong tumor inhibition was observed for combination MM wave - X ray in comparison to separate X ray exposure, and for the next period tumor growth was observed at the same rate. But it should be pointed out that combination MM wave - X ray made much for protection of hemopoietic system if to compare with separate X ray exposure. (fig.5) Group with MM wave - X ray exposure showed karyocyte number was below norm rate only for the first day, and deficit amounted to 20%. At the 5th day, marrow cell level rated at physiological norm. Regeneration of karyocyte number after X ray exposure was observed only by 21st day.

X ray - MM wave exposure caused considerable inhibition of tumor growth in comparison to X ray and combined MM wave - X ray. Upon 7-day course rate of tumor inhibition amounted up to 90-95%. But number of marrow cells by 5th day was decreased. For the first day cell deficit amounted to 30% followed by gradual regeneration.

At double combination inhibition tumor rate by 30 - 35 day reached 90%.

Number of marrow cells after double combined exposure was below norm only during 1 -3 days after finishing of course. Use of double combination MM wave - X ray - MM wave allowed to protect hemopoietic system and to get strong tumor inhibition in comparison to separate X ray exposure.

4. Complex MM wave exposure and antitumor medication cyclophosphan on transplantable tumor sarcoma-180 and hemopoietic system of animals with malignant growth. In 13 days from the start of course with MM wave exposure medication effect on tumor was increased. It was 3-4 times greater than effect of medication with out MM wave exposure and was especially clear indication by 25 day.

Study of hemopoietic system reaction revealed that for animals with complex treatment cyclophosphan - MM wave, on the third day karyocyte number rated at control level and stayed till the end of treatment period (14 days). At action of single medication marrow cell number didn't reach control rate by 14th day.

Encouraging results of the first course of treatment gave an idea to continue treatment. Upon finishing of the second course, since 24th day, an antitumor effect had become evident. (fig.6) By 30th day for animal's group with cyclophosphan treatment only all animals died, and in the group with complex treatment all animals were alive. Rate of tumor resorption reached 90 -100%. Monitoring over the animals had been provided for year and a half. And there were no recurrent cases. The experiment had been repeated four times, each experimental point was estimated as per 45 animals, total for testing - 180 animals.

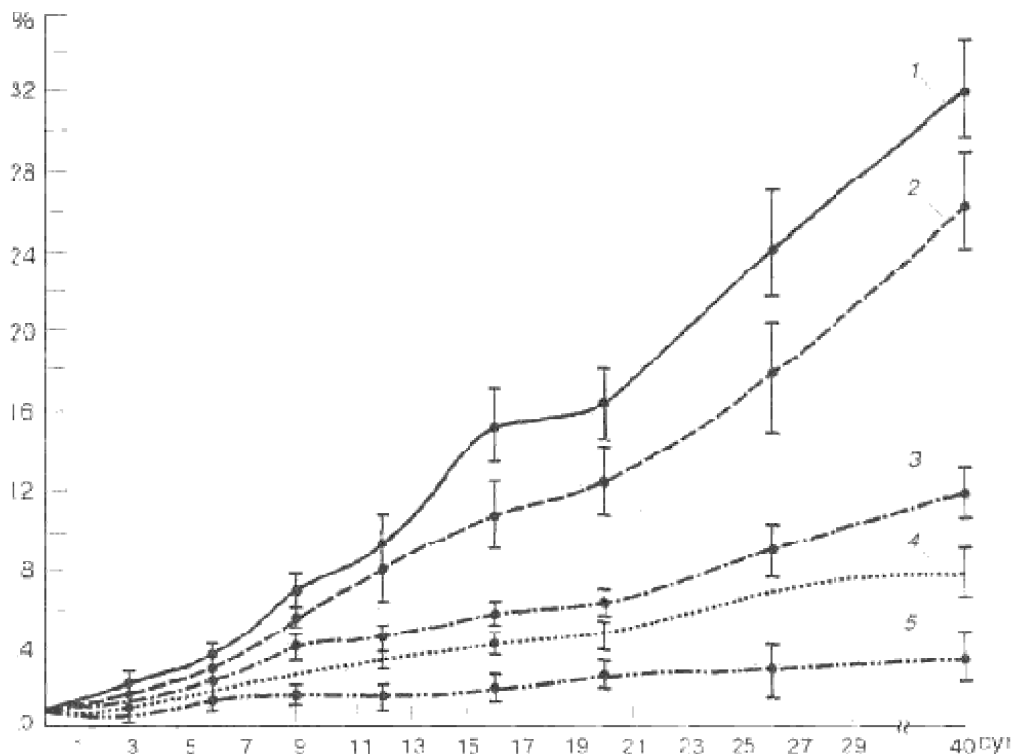




Fig.5. Tumor growth inhibition (sarcoma-45) at combined action of MM wave and X ray, X ray and MM wave and X ray only: 1 - control, animals with tumor; 2 - tumor exposure by MM wave only; 3 - tumor exposure by X ray only; 4 - combined exposure MM wave and X ray; 5 - combined exposure X ray and MM wave. Abscissa axis- time of action (in days), ordinate axis - percentage of tumor growth inhibition.

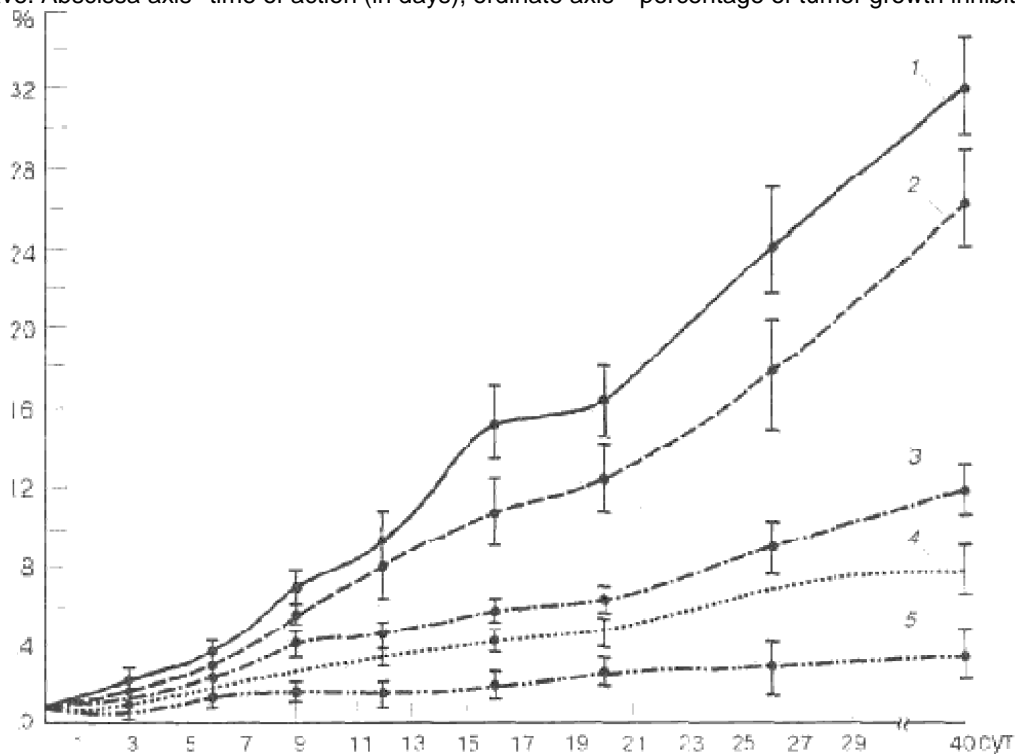


Fig.6. Tumor growth inhibition (sarcoma-45) at complex action of cyclophosphan and MM wave : 1 - control, animals with tumor; 2 - hip exposure by MM wave only; 3 - cyclophosphan only; 4 - complex action of cyclophosphan and MM wave. Abscissa axis - time of action (in days), ordinate axis - percentage of tumor growth inhibition.

Investigation of erythrocyte dynamics for animals under two courses found out that combined treatment ensured erythrocyte protection during the whole treatment. For group of animals with combined treatment counting of erythrocytes had been provided by 51st day, and its number framed in norm.

Thus, combined treatment MM wave - cyclophosphan on animals with sarcoma-180 contributed to decrease of preparation toxicity and at the same time potentize its antitumor effect.

Influence of MM wave exposure on hemopoietic cells of marrow had been revealed during experiments in vitro. Study of Ignasheva L.P. and Soboleva /23/ describes investigations on survival of lethal irradiated mice after transplantation of crio stored marrow exposed by MM wave upon unfreezing.

Myelo transplantation success depends on degree of functional safety for stem hemopoietic cell. Usually regeneration of marrow haemopoiesis for animals being transplanted with unfreeze marrow takes place at 708 days later than in case of fresh marrow. One of reliable methods for marrow cell vitality estimation is survival (over 30 days) of animals exposed by lethal doze.

Hybrid mice were used as donor and recipient.

Crio stored marrow cells had been exposed by MM wave with wavelength 7,1 mm at optimal program regime.

Animals in control group died by 15 day without transplantation of acute radiation sickness with evident clinical presentations: loss in weight, adynamia, and hair thin out. For animals in group with transplanted unfreeze marrow but without exposure survival made up 45% by 30th day, and, at the same time, for animals with transplanted unfreeze marrow exposed by MM wave survival made up 53% - the highest index.

As for clinic symptoms for animals both groups showed minor loss in weight with regeneration tendency by the end of the observed period.

So, low intensity MM wave exposure exerts positive influence on stem hemopoietic cells of crio stored marrow, promotes high vitality of lethal radiated recipients upon myelo transplantation and could be one of ways of repopulation ability increase for crio stored marrow.

Institute of Radiotechnique and Electronics of Russia Academy of Science and Moscow Research Oncology Institute by P.A. Gertzen since 1989/90 are carrying out experimental research on low intensity

microwave pulse of nanosecond duration with high peaking capacity exposure interaction on malignant tumor [24,25]. Despite of high exposure power, there is practically no heating effect on the object due to short pulse duration (about 10 nsec). At the same time the radiation doesn't ionize the object and doesn't break molecular coupling, since the energy of electromagnetic quantum radiation within the wavelength band is rather low. A characteristic feature for the said pulse exposure is high electrostatic intensity, which can be compared to natural quasi-static intensity of electric field at biological cell membranes.

Experiments had been performed on mice of Wistar line with intramuscularly transplantable Walker's carcinoma sarcoma.

In result of numerous experiments with pulse MM wave and UHF wave exposure described above the following features for exposed animals had been revealed in comparison with the control group:

- Increase lifetime
- Decrease of speed growth and stabilization of transplanted tumor (on a few days growth is stopped)
- At combination of drug chemotherapy and mm wave exposure tumor growth is effectively slowed down and lifetime is prolonged.
- Degree of metastatic affection is decreased both for combined mm wave / UHF exposure and for separate exposure.

As for experiments in vitro, it was revealed increase of number of tumor cells for different stages of cell destruction up to total cell death in comparison to non-exposed cell suspension. Healthy animals being exposed 1 - 1,5 years ago, didn't revealed any visible changes neither in behavior reactions nor in general condition. Autopsy of exposed animals didn't found out any pathology changes in liver, kidney, epinephros, and immune competent organs (thymus, pancreas, glands) in comparison to control aging group.

Consequently, grounded on the provided research, we can report about direct action of pulse radiation on tumor cells and about indirectly action - through activation of immune system.

Group of scientists under guidance of Govallo V.I. at Scientific research Institute of Traumatology and Orthopedy had conducted investigations on influence of electromagnetic radiation of MM wave band to human lymphocytes and fibroblasts. /26/.

It was shown that under MM wave exposure lymphocytes and fibroblasts generated phytoquin factor, which increases growth and functional activity for similar cells. Phytoquin is contained at high concentration in destroyed exposed cells, its lysates and also liberated into cultural environment. MM wave exposure itself didn't activate cell growth, didn't change expression of surface lymphocyte's receptors and didn't influence to its sensitivity to mitogen or exogenous immunomodulators, but addition of phytoquin into culture environment sharply stimulated proliferative potential of lymphocytes and fibroblasts.

The said factor, phytoquin, which is produced in cell's cytoplasm is connected with dehydrogenase's activation: in exposed cells concentration of lactate dehydrogenase is increased in 3 - 5 times. Activating factor corresponds to category of cell regulators - cytokines. It doesn't belong to the interleukin or interferon group, but can be lymphoquin or monokine. It is a low-molecular glycosylated factor, which secreted locally and distantly and which is acted as paracrine or autocrine (but not as endocrine).

Apparently, the described mechanism can be named as fundamental for immunopotentiating effect of mm waves. Such effect had been observed at treatment of septic wounds and complications. Difficulties at treatment of such diseases can be related with insufficiency of organism's immunity, with long-term and complicated operations, with changing of properties and character of purulent infection, which reveals resistance to many antibacterial medicines applied, with increased trauma complication, etc.

At application of MM wave exposure for treatment of severe types of war pathology (mine explosions and gunshot wounds) for locomotorium, accompanied by purulent-injured infection the following results had been revealed:

- Wound process duration for different phases, including recalcitrant wounds had been reduced in 1,5 - 2 times in comparison with the control group;
- Apparent stimulating action of MM waves to tissue regeneration in the wound area - degree of wound surface per day corresponded to the same manifestation for noncomplicated wound;
- 100% degree of engraftment for transplant;

- Stop of osteomyelitis process: no pains, no inflaming for injured arm segment, full or partial recovering for fistula, soft wound epithalisation;
- Satisfactory prognosis in postoperative treatment period for 92,3% cases;
- 20% decrease of recurrence after surgery;
- Decrease of wound microbe semination after purulent necrotic opening and excision.

Microbiology research in vitro on MM wave influence to biochemical and cultural properties of microorganisms, microbe sensitivity to antibiotics hasn't established direct action of MM waves.

According to investigations, the major contribution of MM wave action is reached by normalization of immune parameters. Patients with complicated surgical operations are often suffered of secondary immunodeficiency, and it is a strong interfere factor. Application of MM wave action positively resulted to immune gram: increase in content of relative and absolute T -cells (at 30 - 80%); T-helpers increase (at 30 - 80%), increase of GBL or EK-cell content (at 40-60%) , Immune Reactive Index (IRI) normalization and LTI decrease.

Accordingly, antiinfection action of MM wave exposure is realized not directly on pathogenic flora but due to increase of general resistivity of organism and tissue viability in wound area.

As an example of profound immune simulating effect of MM wave action we are listed the work of St Petersburg scientists on prophylactic action of MM waves at grippal infection.

In the first series of experiments it was shown a protective effect of MM wave at application of Grippe virus A both for infected animals and for animals exposed before infection:

- All experimental groups revealed positive prophylactic effect both as for survival and for average life span;
- Degree of clinical apparent protective effect depends on exposure method - the best effect (zero death-rate) is observed at prophylactic exposure of healthy animals before infection;
- Prophylactic effect increases with prolonged prophylactic period from 7 days up to 17 days;
- Sufficient effect is observed at application MM wave exposure as therapeutic agent.

Along with experimental research on animals it was conducted a retrospective study analysis of epidemiologic situation on influenza and acute respiratory disease for patients with mm wave treatment of stomach ulcer. Course of mm wave therapy took place at influenza (virus A) pre - epidemic period. Patients being treated with mm wave therapy, in comparison to control group (similar as per age, health conditions and labor conditions). In result it was revealed that patients with mm wave therapy had 1,78 times lower sickness rate than patients from control group.

It is known that most diseases are connected with secondary immune deficiency, and immune stimulation action of mm waves is of great interest.

Treatment of Stomach ulcer is connected with regeneration of protection and aggression factors for the whole organism. And immunity is at the first place among such factors. At comparison analysis at treatment of stomach ulcer using mm wave and using medication therapy [29], non specific immunity ( phagocytosis, lysozyme) and specific immunity ( T -, B-lymphocytes, immunoglobulins A,M,G) had been studied . At medication therapy despite of ulcer healing no protective factors were revealed. At mm wave therapy along with ulcer healing without colloidal scarring it was revealed marked stimulating effect both for specific immunity and for non-specific immunity. At dynamic monitoring for patients with mm wave therapy it was found out that protective factored reached its maximum after three months upon treatment. Considering that mm waves normalize protective factors of the organism, it was suggested prophylactic mm wave therapy.

At treatment of atopic dermatitis [30,31] with application of mm wave therapy, the following immune parameters had been testes for control:

- Detection of T-lymphocytes - "active" and "general";
- Detection of B-lymphocytes;

- Detection of A,M,G immunoglobulins in blood serum by radial immune diffusion method in agar gel;
- Detection of circulated immune complexes (CIC) for blood serum;
- Immune fermentative analysis for detection of common IgE and allergen specific IgE - antibody to grass pollen, household and food-borne allergen.

In result of treatment it was marked positive dynamics and persistent improving of immune parameters both for cell immunity (Ta-ROC, E-ROC) and humoral immunity (CIC, IgM, IgC, IgE).

Patients with medicated therapy didn't show any changes neither for cell nor for humoral immunity. Group of authors from CSRI of tuberculosis Russian Academy of Science [32] had studied influence of mm waves on immunity at therapy of pulmonary sarcoidosis: it was detected T-lymphocyte number and its functional activity with FGA, B-lymphocyte number, immunoglobulin rate and CIC for blood serum before and after treatment. Universal stimulation of functional activity for immune competent cells had been observed: activation of macrophage phagocytic function in granulomatosis area, in distanced lung areas, in blood. Due to macrophage activation CIC released from organism more quick and effective, its phagocytosis by macrophage, which content decreased after mm wave therapy for 87-91% of patients. It resulted to lung bloodstream reduction - it is known that decrease of CIC number in blood protected microvascular damage for many organs.

Herpetic infection is often marked for the resent time, it happens due to inadequate prophylactic and non-sufficient medicated therapy as well as with growth of ecological immune deficit, wide use of antibacterial and hormone agents. At complex treatment comprised of medicated therapy and mm wave therapy, at examination of immune status (T-, B-lymphocytes, CIC, immunoglobulins A,M,G, immune modulators tolerance) it was revealed immune stimulating effect, evidenced in activation of phagocytosis and T-lymphocytes, which is of great importance for diseases treatment accompanied with secondary immune deficit [33].

Nowadays it is also widely spread urogenital infection both for men and women, often initiated by clamidia, microplasma and ureaplasma infection. Such infections often accompanied with secondary immune deficit. Antibacterial therapy accompanied by immune stimulation increases success percentage up to 70% in comparison to 30-50% of usual therapy[34].

It is known that acne rush often appears at suppressed immunity. A study on mm wave therapy influence on acne vulgaris had been conducted. Immune profile had been taken for all patients, detecting cell and humoral immunity before and after treatment. Patients, which had shown recovery of immune parameters, didn't have growth for skin conditional pathogenic microorganisms. Such resulted were considered as clinical recovery. As a whole, for all patients it was found positive dynamics of immune parameters accompanied by considerable improve in microorganisms skin microbiocenosis[35].

Accordingly, provided clinical-experimental study testifies to clinically apparent immune modulating effect of low intensity mm waves.

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A novel and selective physical anticancer agent.

"Experimental"

ABSTRACTS

The effects induced by low power millimetric waves (MMW) radiation on the growth of tumoral and healthy cells have been studied. Wide-band frequency range between 53.57-78.33 GHz with radiation density power of  $2.7 \cdot 10^{-17}$  watt/Hz have been used. The radiating energy was such low as not to increase the temperature of the cellular samples (cold irradiation). One hour of radiating treatment made every other day over three tumoral human stable cell lines, produced a noticeable depression of the cellular growth. The analogous treatment made over two healthy cell lines, gave a weak growth stimulation.

Scanning electron microscopy study of MCF-7 and K562 irradiated cells reveals that MMW irradiation induces profound morphological changes of their membrane. Finally, we also provided a mechanistic indication, based on millimeter wave spectroscopy of the cells: water is the primary absorber of these electromagnetic waves. Our work gives interesting evidence that wide band low power MMW irradiation, in the appropriate frequency range could be used in future as a cold mean to cause a selective depression of tumoral cells growth.

INTRODUCTION

The study of the interaction between millimetric waves (MMW) and biological systems began with the pioneer work of Webb and Dodds who, in 1968 described the MMW influence on Escherichia Coli bacterium cell growth (1).

Afterwards, in 1974, experiments by Devyatkov (2) and by others (3-7) demonstrated that the interaction between MMW and biological systems in the 39 to 60 GHz range, is frequency dependent. From these studies some important indications, concerning the interactions between MMW and biological systems, have emerged. In particular, it seems possible to say that:

- a) low power MMW and the biological system can have resonant interactions;
- b) in order to have an effective interaction the MMW power must be above a limiting value;
- c) the effect of MMW on biological systems depends on the irradiation time.

Some hypotheses have arisen regarding the mechanism which might be involved in producing these effects (8). One of the first ideas given by Frohlich regards the implication of non-linear vibration excitations (9-12). Keilmann (13), in 1986, proposed that the resonant biological effect of MMW could be

associated to low frequency electronic transitions of transient triplet state molecules. The resonant interaction of the microwave with the multiplet transitions would equalize the populations of the substates, which are originally assumed far from thermal equilibrium. This mechanism would change fundamental molecular properties such as the molecular metabolic reaction rate, thus inducing great variations into their biochemical pathways.

Despite the fact that interaction mechanisms between MMW and biological systems are not yet understood, the experimentation on MMW application in biology and in medicine is gradually becoming more and more important.

A recent review of the most significant publications concerning the effect of MMW on cell free systems, cultured cells, isolated organs of animals and humans is due to Pakhomov et al (14).

In animals and humans, the local application of low power MMW irradiation is producing important clinical benefits such as: tissue repair and regeneration, alleviation of stress reaction, better recovering in a wide range of diseases (14).

The low power MMW irradiation is surely becoming an important clinical tool especially in Russia where MMW bands comprised in the range between 35-75 GHz are in the therapeutic treatment of lung diseases, bone arthritis, heart disease and gastric ulcer (14).

For what being in our knowledge and on the basis of the above mentioned literature there is a widespread conviction that low power MMW, in the frequency range between 30 and 68 GHz, in athermic conditions, behaves as a sort of cellular growth factor.

From these premises, we have been stimulated to begin the study of the effects of low power MMW on tumoral cells, in order to ascertain the possible role of this part of the electromagnetic spectrum on the proliferation of human tumours.

We compared the effect of MMW on healthy human cells, monitoring them in analogous conditions.

The investigated tumoral cells were:

- a) MCF-7: a stable cell line of breast carcinoma;
- b) K562: a stable leukemia cell line;
- c) primary cell line from a breast primary tumour.

The non tumoral human cells were:

- d) normal human breast epithelial;
- e) normal human lymphomonocytes.

#### MATERIALS AND METHODS

Experimental MMW facilities and culture cell methods. The radiation experiments have been carried out through instruments produced by the UWOM company of Nizhny Novgorod (Russia). The MMW radiating apparatus, the AMFIT 32, is constituted by a microwave noise generator dyod, and an opportune wave guide that is able to supply a frequency band 53.57 -78.33 GHz. The peak density power radiated by this instrument is 2.7 10<sup>-17</sup> Watt/Hz. All cellular systems, located in 35 mm polystyrene Petri dishes and supplemented by 2.8 ml of the aqueous culture medium (layer thickness was 2.9 mm), have been radiated directly on the open surface of the culture dishes which were placed at a 18 cm distance from the conical antenna of the instrument. In this condition the radiating density power reaching the sample was of the order of 0.07 Watt/cm<sup>2</sup>. It can be argued by simple energetic considerations, that such radiation power can not induce changes in the sample temperature, as has been experimentally proved. AMFIT 32 is only able to radiate in continuous wave mode and has been used in this work to carry out experiments on MCF-7, K562, primary human mammary tumoral cells, human lymphomonocytes and healthy human epithelial mammary cells.

#### Cell Cultures

MCF-7 human breast cancer cells (15) were obtained from American Type Culture Collection and cultured in low glucose Dulbecco modified Eagle's medium (DMEM; Sigma), supplemented with 7.5% fetal calf serum (FCS) and 50U/mL penicillin and 5g/mL streptomycin at 37 °C in a humidified atmosphere with 5% CO<sub>2</sub> and 95% air. Cells from stock flask were suspended by treatment with trypsin, buffered with phosphate buffer saline and counted using a hemocytometer. Each culture was seeded into 35 mm plates, at a concentration of 5.0 \* 10<sup>4</sup> cells/ml in 2.8 ml of medium. After about 3 days from seeding active growth of cells began and only after this period we started the microwave irradiation.

This cell line has a doubling time of 48 hours.

K562 cells, chronic myelogenous human leukemia, obtained from the "Centro Studi della Microcitemia", (Cosenza), were grown at 37°C in Roswell Park memorial Institute (RPMI 1640), from Sigma, supplemented with 10% (V/V) fetal calf serum, 50U/mL penicillin, 50g/mL streptomycin, 20 mM Hepes and 0.85 g/L NaHCO<sub>3</sub> to adjust pH values from 7.2 in a fully humidified atmosphere of 5% CO<sub>2</sub> and 95% air. Cells from stock flask were seeded at a concentration of 1.0 \* 10<sup>5</sup> cells/ml in 2.8 ml of medium into 35 mm plates. This cell line has a doubling time of 26 -30 hours.

Normal and neoplastic mammary epithelial cells. Mammary epithelial cells were obtained from normal mammary and primary breast cancer tissues by enzymatic dissociation as described (16) with some modifications. Primary tumoral and adjacent normal tissue samples were derived from mastectomy specimens from a patient with infiltrating ductal carcinoma and histologically characterized. The tissue



specimens were placed in sterile tubes containing medium 199 with antibiotics and transported to the cell cultures laboratory. The tissues were minced with scalpels, after removal of fatty areas and blood clots, and incubated overnight in the enzyme digestion mixture (medium 199 with 150 U/ml type III collagenase and 0.6 mg/ml dispase). Enzymatic digestion was performed at 37°C with gentle agitation by a shaking water bath. The clumps of cells obtained by the digestion were collected by filtration with 70 µm pore size polyester filters. Both the filtrate and the organoids were seeded into culture. The medium consisted of Ham's F-12 supplemented with insulin (5 m/ml), hydrocortisone (1 g/ml), EGF (10 ng/ml), cholera toxin (100 ng/ml), 5% FCS, penicillin and streptomycin (17 -29).

Active growth of epithelial cells began about 4-5 days after seeding. Fibroblasts were removed from epithelial cell colonies incubating the cultures for 2-3 min. at room temperature in trypsin-EDTA.

Human lymphomonocytes. Different samples, coming from two healthy blood donors were analyzed.

Lymphomonocytes need mitotic agents to grow, so that we supplemented the medium with phytohemagglutinin (PHA 2 ml/2.8 ml) as described by Perper (30). The experiment wished to show the effect of MMW irradiation on lymphomonocytes growth. Of course, as far as the irradiation conditions are concerned, they remained the same as those used in the experiments described above (the spectral density power was 10-17 watt/Hz; the irradiation time was 1 hour every other day, starting from the seeding).

#### Scanning electron microscopy

MCF-7 cells were directly seeded on glass supports for SEM analysis, placed inside a petri-dish, at a  $5 \times 10^4$  cells/petri final concentration. MMW irradiation, carried out under the same condition used in the growth experiments, started after the cells were adhered on the SEM support that was pretreated with a 0.1% w/v poly-L-lysine solution. This procedure was required to avoid trypsinization and centrifugation steps that could alter the cell morphology. After the last irradiation (4th), cell monolayers were rinsed twice with phosphate buffer solution (PBS), fixed with 5% glutaraldehyde solution (pH 7.4) for about 2h at 4°C, again, rinsed twice with PBS and post fixed with 1% Osmium tetroxide solution (pH 7.4). They were then dehydrated in a graded series of alcohol for 5 minutes each, critically point dried using a K850 CPD EMITECH and coated with a thin layer of gold-palladium using a K850 SPUTTER COATING EMITECH.

The specimens were observed using a ZEISS DSM 940 microscope.

K562 cells were seeded in multiwells at a final concentration of  $5 \times 10^4$  cells/well and irradiated with the same irradiation protocol adopted in the previous experiments. After 4th irradiation, cell suspension were diluted with phosphate buffer and seeded on the SEM supports where they were treated for the SEM observation in the same way as described for MCF-7 cells. This procedure does not need centrifugation thus avoiding possible cell morphology alteration.

Mechanistic study: millimeter wave spectrometer.

As it is shown in the block scheme in fig.1, our millimeter wave spectrometer is constituted of a sweep generator (a backward wave oscillator, BWO) of the MMW in the 50-80 GHz range. The BWO generates a power ranging from 0.01 to 3 mW. By a suitable waveguide, the electromagnetic wave is propagated towards the sample allocated in the measurement camera, fig.2 (C). The latter is a portion of an infinite rectangular waveguide in which a narrow centered slot is cut on its broad side (fig. 2; L = 0,32 mm thick).

This provides a suitable point of entry for a probe used to sample the interior waveguide field. The samples were inserted between two slabs of PET (polyethyleneterephthalate) used as a probe (fig.2; S).

In this way we were able to detect the MMW power reflected by samples such as aqueous solutions (water and culture medium), cellular suspensions (K562) and also as adherent type cellular system (MCF7). In this way we avoid the strong decrease of power due to the resistive absorption of water. PET in fact, is a good substrate for the growth of MCF7 cells. Furthermore, PET is transparent to the MMW used, a necessary requirement to perform the reflection experiment.

In order to avoid possible overlapping of the waves reflected from the sample and those reflected at the end of the waveguide, a wave-absorber was placed after the camera. The wave reflected by the sample and the reference wave reaches respectively detector 2 and detector 1. The detector is able to rectify the MMW and then, to transmit a continuous signal to the receiver. The two signals, coming from the detectors, are amplified and compared in the receiver. The difference between these two signals is then digitalized by an analogical converter unit and the reflection coefficient is displayed as a function of the frequency. The digital unit of the instrument, allowed us to store the spectra and to mediate between more spectra, thus increasing the sensitivity of the measurement.

#### Results.

We describe the experiments done on tumoral and non tumoral cells by using continuous wave (CW) MMW. In all cases the irradiation started at the active cellular growth phase.

Determination of the total cell count and viable cell number were made by the use of the hemocytometer and trypan blue stain. Each point in the growth curves represent the number of viable cell. The count were made in duplicate. Standard deviation is of the order of 7%.

Adherent cells (MCF-7, neoplastic and normal epithelial cells) were irradiated after 3 days from seeding while non adherent cells (K562 and human lymphomonocytes) were irradiated after the seeding. The CW radiating treatment was done for 1 hour every other day and the cells counted every day in duplicate. The

growth in the irradiated samples is respectively depressed in MCF -7 cells of about 60%, in K562 of above 50% and in neoplastic mammary epithelial cells of about 40% with respect to the non irradiated ones. Non tumoral cells (normal mammary epithelial cells and human lymphomonocytes) irradiated in the same experimental conditions of tumoral ones had an increase (~15%) in the irradiated population.

The trend shown were confirmed in at least four repeated experiment.

#### SEM results.

Photos in fig.5 illustrate the surface morphology of MCF -7 cells respectively before and after MMW irradiation. Under physiological conditions, MCF -7 cultured cells present, on their surface membrane, structures like microvilli that are protrusions of the plasmatic membrane. This is a characteristic feature of all epithelial cells (fig.4a). MMW irradiation causes a noticeable decrease of the number of microvilli, as can be observed in photo 1b that represent the morphological status of about 80 % of the irradiated cells.

Many K562 cells were rounded with a narrow ridge-like profile and present a ruffled membrane. Occasionally, they had a smooth surface or present surface blebs. In the irradiated sample, most of the cells (about 90%) were rounded with a smooth surface.

#### Spectral results.

We reported the spectrum of K562 cells with the spectra of water and RPMI culture medium. In the case of MCF-7 cells we obtained the same result.

The spectral profiles shown in fig.7, are only apparently different. Due to small differences in the thickness of the samples, reflection measurements, from different sample, may be affected by random offset and magnification effects. In order to show that the three spectra reported bring the same information, we also report the spectral differences obtained by the equation

$$d(w) = S1(w) - [a + b * S2(w)]$$

where,  $S1(w)$  and  $S2(w)$  are spectrum 1 and 2 respectively;  $a$  is an offset parameter for reflection and  $b$  is a parameter that shift the global amplitude of spectrum 2. When  $a$  and  $b$  are properly adjusted,  $d(w)$  for the three possible differences, goes to zero in the limit of the experimental error (fig.7 on the right).

Any way, it is worth to note that the spectral profiles show distinct reflection peaks between 52 and 65 GHz.

#### Discussion.

Our experiments show the influence produced on several cell systems by MMW radiation having a global power below 0.1 mWatt/cm<sup>2</sup>. The temperature of the investigated systems remained strictly controlled and stable at 37 °C. Due to the very low power used in the experiments, the radiation has never produced detectable thermal effects. In spite of this fact the radiated systems were for the most part strongly influenced. Of great importance, in reference to the biomedical application of low power MMW, is the fact that such radiation can produce a considerable decrease of tumoral cell growth. In analogous radiation conditions, healthy human cells are not negatively affected, rather, in some cases cell growth is actually stimulated (15% of growth increase in the case of healthy mammary epithelial cells).

To further investigate the cellular response to MMW waves we perform the electron microscopy study starting from SEM observation of the irradiated MCF -7 and K562 cells. Such morphological analysis provided us strong mechanistic indication about the MMW -cell interaction. Actually, the alterations observed on either MCF -7 and K562 plasmatic membranes involve some functional variations of the membrane properties that could be correlated to the observed effects on growth.

We made a first, important step in the comprehension of the mechanism of interaction between millimeter waves and biological systems, building a millimeter wave spectrometer able to measure the reflection produced by dielectric material irradiated by the electromagnetic waves in the 50 -80 GHz frequency range, the same range used in the growth experiment described above.

By the use of this spectrometer we were able to determine the behaviour of the reflection coefficient of dielectric sample (biological system) as a function of the frequency of the electromagnetic wave.

Our observations show that, in the 50-80 GHz frequency range, the absorption phenomena presented by cellular systems, is entirely due to the absorption of water.

This conclusion is in accord with earlier studies on the relaxation mechanisms of biological systems (31 - 32), in which it has been determined that at frequencies above 1 GHz, the dispersion region of the dielectric dispersion curve of various biological systems is caused only by the absorption of water.

Furthermore, our results agree equally well with the recent work of Devyatkov et. al (33). These authors irradiated water and human tissues with MMW in the 40 -100 GHz range and observed the response of the two systems in the radiofrequency spectrum. They have shown that the stimulation of such systems with three different MMW restricted bands causes the emission of three well defined peaks power in the decimetric range of electromagnetic waves. Surprisingly, the excitation and response bands were identical for both water and human tissues systems.

To explain these results, the authors proposed that MMW must, first of all, interact with the water dynamic. At some excitation frequencies, the interaction mechanism implies resonant phenomena that extends over a large number of water molecules (large sample volume). This collective oscillations that occur in the system, induce the emission of decimetric waves.

The morphological alterations observed, thus, can not be induced by direct interaction of MMW with the membrane, but they have to be mediated by water. Our recent findings show that when we irradiate a model membrane as n-dodecylpentaoxiethylene/H<sub>2</sub>O lamellar lyotropic liquid crystal, we induce an increase in the amount of water bound to the membrane and this involves a membrane rearrangement (submitted for publication by Chidichimo G, Beneduci A and Filippelli L). At the present stage we are then led to attribute the important effects induced on tumoral cells by low power MMW radiation, in the 50 -80 GHz frequency range, to variations of the cellular membranes due to the resonant absorption energy of the water molecules acting as receiving antenna.

Even if further investigation is needed to clarify all biochemical effects induced by MMW on cellular systems, we believe that these preliminary results open a very interesting biomedical research line which is linked to the possible use of low power MMW radiation to selectively strike tumoral cells, without damaging other tissues.

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## Bacteria



Nowadays development strategy retrieval on plants agriculture production supposes application of different technologies. Along with widely discussed and applicable technologies on the basis of genetically transformed plants, methods of complex bacterial agents is also of great interest, allowing to get safe plant product, balanced at aminoketone rate, carbohydrate and mineral composition.

Plant and animal nutrition depends on bound or fixed nitrogen sources. Bound nitrogen in ammonia form, nitrate form, organic compounds is of relative deficit in water and soil and often limits growth of living organism. There is an inexhaustible supply of gas nitrogen in the atmosphere (80000 ton above 1 hectare), while bound forms of nitrogen are of relative deficit on the Earth surface. Due to that fact one may suppose that the forming process of bound nitrogen is a weak point in global cycle of nitrogen - an urgent biogenic element. Over 90% of bound nitrogen is formed at the Biosphere in result of activity of nitrogen fixing microorganisms. According to the materials of the SCOPE (Scientific Committee on Problems of the Environment) nitrogen is being fixed by bacteria up to  $130 \cdot 10^{15}$  gramm/year in the Ocean and up to  $260 \cdot 10^{15}$  gramm/year at the Surface (Meadows et al., 1972; Stanier et al., 1976; Bolin et al, 1983).

Agriculture planting strongly depends on presence of bound nitrogen forms and its accessibility in the soil. Usually applied nitrogenated fertilizers (ammonia, ammonium nitrate, etc) are rather expensive or in deficit. Besides, the major part of fertilizers is quickly moved off the soil by underground waters ( $\text{NO}_2^+$ ,  $\text{NO}_3^+$ ) or evaporated as gas compositions ( $\text{NH}_4$ ) (Genkel, 1974; Stanier et al., 1976; Postgate, 1982). So, plants can assimilate only a little part of bound nitrogen, which cause additional costs for forming a sufficient nitrogen concentration in soil. At the same time, an excess of nitrate ( $\text{NO}_3^+$ ) or nitrite ( $\text{NO}_2^+$ ) concentration courses nitrate pollution of plant products, underground waters and freshwaters (Genkel, 1974; Stanier et al., 1976). Manufacturer is faced with the dilemma: whether to keep high nitrogen concentration in soil, which could effect environment and product pollution, or to get poor but "clean" crops.

The problem could be easily solved by nitrogen fixing bacteria. Usual environment for bacteria is on the root surface (rhizoplane) or just in close surroundings (rhizosphere); bacteria enrich soil with bound nitrogen forms just in close surrounding of roots. At the same time, associative relations of the plant and microorganisms regulate the number of microorganism's nitrogen fixing cells and its activity (Stanier et al., 1976; Gromov, 1989). Granting this it is possible to support fixing nitrogen at adequate constant rate in the soil.

Nitrogen-fixing activity of the nitrogen-fixing microorganisms is directly connected with common physiological activities of bacteria (Kurz et al., 1975; Gottschalk, 1979). Activity of the nitrogen -fixers increases if they live in association with the plant: in rhizoplane or in rhizosphere. Nitrogen-fixing effectiveness is strongly influenced by plant: plant supplies bacteria with the required energy substrates. It is shown that root exudation (extraction) along with vital root dropout could amounts up to 50% of the total quantity of photosynthesis products per vegetative period (Trinkick, 1973; Babyeva I.P., Zenova G.M. 1983).

Bacterial agents are products of nitrogen fixing microorganisms, mostly *Rhizobium* bacteria are successfully used in agriculture practice. First, bacterial agent was made in Germany in 1896 from tuber bacteria and was called nitrogen. In 1906 an agent was manufactured in England, in 1907 - in USA. In Russia agent was manufactured in 1902, and in 30h - the first mass production (Mishustin, Shilnikova, 1968; Genkel, 1974). An important feature of bacteria agents on the basis of diazotroph is its physiological activity. A positive feature of physiological activity is indolyl-3-acetic acid - the major stimulator for cell growth, so called growth hormone and some other active compounds (Umarov, 1986; Gromov, 1989; Wipps, Lynch, 1986; Olunina and others, 1999).

Manufacture of nitrogen fixers activated cultures is an urgent target of applied microbiology. One of available pollution free adequate methods for stimulation of physiological activity of microorganisms is processing of cell population by short wavelength electromagnetic field. (Low intensity electromagnetic field of mm wave range).

Action of mm wave radiation has been being studied for the last 25 years on different bio objects (from bacteria up to tissues and human organs) and model systems, and as well as application in medicine, which has turned to foundation of EHF therapy. Recently, on the basis of numerous works on mm wave action to living systems a new trend has been created. We are speaking about mm wave action to photosynthesing objects and reaction centers of photosynthesing bacteria (Tambiev, Kirikova, 1995). Su rvey on mm wave action to bio objects evidences of mechanisms of mm wave interaction with cells of plant and animal nature, which touches fundamental aspects of vital activity. Common features and mechanisms of such interaction have been investigated and s tudied as well as various physiological effects of low intensity EHF radiation: growth acceleration and bio mass increase, photosynthesis intensification, accompanied by oxygen separation and increase of photosynthesing pigments content, speeding of organic excretions into the media, reactivity of examethabolits changing, ion transport changing and others. Similar effect was observed at mm wave action to cyanic bacteria, and active nitrogen fixers are among them (Tambiev, Kirikova, 1994, 1995, 1998, 2000).

Results of these researches allow to ensure progress in physiological activity stimulation for other microorganisms, and as well as diazo troph bacteria.

Nizhny Novgorod State University Chair of Molecular biology and Immunology has been providing re search on bio agent design for seed preplant processing. There is a collection of culture for nitrogen fixing bacteria being adapted to domestic agriculture plants. Nitrogen fixing culture forms bacteria agents' basis for vegetable seed preplanting for fields and conservatory. All the cultures characterized by ability to indolyl -3 acetic acid secretion. Preparations being investigated on these cultures have been tested by Scientists of Agriculture Academy on different crops. It was clearly defined effect of seed preplanting for cucumbers and tomatoes on increase of fruit biomass as well as enlarging crop capacity of the cereal crops for 5.8 -7.4 centner /hectare, at average capacity 30 -36 centner /hectare (Ladygina, Olynina, Rechkin, Alekseeva, 2002).

The target of the project is experimental research on application of low intensity mm wave radiation for activation of physiology activity and yield of biomass of nitrogen fixing bacteria, which applied for bio agents engineering.

It is supposed to perform research on optimization of mm wave radiation parameters for the following goals:

- To increase biomass output of nitrogen fixing microorganisms;
- To activate secretion of plant's growth hormone;
- To test received agents during field investigations.

We are looking forward to get the following gains:

Active cultures of nitrogen fixing microorganisms will allow creating of bio agents adapted to specific regional (landscape) conditions, which will give the following benefits:

1. To keep stable concentration of bound nitrogen forms for cultivated plant's rizosphere at the required rate by minimum costs for nitrogenated microorganisms.

2. To cut down expenses for additional processing of vegetating plants by pesticides (as purposed processing) and nitrogen additional fertilizing of plants, and also by forming competitive microflora in contrast to pathogenetic agents of rizosphere microflora. To enlarge products shelf life and volume of safety goods.

3. To improve quality of vegetating products as per protein content in result of plant full -value feed with bound nitrogen forms over the whole vegetation period.

Possible users.

1. Hothouse planting, specialized at vegetable planting - tomatoes, cucumbers, cabbage, beet.

Application of the agent will allow to get qualified pollution free production with valuable content of bio elements, including protein as well, with improved resistance to viruses and bacteria infections, affected fruits.

2. Vegetable manufacturers for open soil - preplanting processing of potato tubers, seeds and seedling. The agent processing allows improving seed germination, growth stimulation, its resistance to phyto pathogenetic organisms and action of adverse natural factors.

3. Individual manufacturers of agriculture goods - farmers, gardeners, phyto designers and others.

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