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Full Length Research Paper

The effect of MRET activated water on staphylococcal infection *in vivo* in animal model and *in vitro* on the culture of *Staphylococcus aureus* wood-46

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The article relates to detailed observation of the effect of MRET activated water with the modified molecular structure on *Staphylococcus aureus.* MRET water is produced with the help of Molecular Resonance Effect Technology patented in the USA. The investigation described in this article was conducted in animal model and *in vitro* at the Division of Microbiology and Immunology, Biological Department of Kyiv National Shevchenko University, Ukraine. The research in animal model revealed the fact that the consumption of MRET water stimulated the phagocytic activity and the immune response. The phagocytic system is one of the main factors of natural non-specific cellular resistance to infections and inflammations. It is the first line of protection of an organism against the penetration and reproduction of pathogenic microorganisms. The protective role of phagocytes is based on their capacity to identify, engulf and neutralize the alien agents penetrating into internal environment of a macro - organism. Particularly, the consumption of MRET water reduced the death rate from 30% (control group of mice on non-activated water) to 0% (two groups of mice on MRET water) during the first 9 days of experiment after intra-peritoneal inoculation of *Staphylococcus* culture. The significant bacteriostatic effect of 70 - 100% (depending on initial concentrations of pathogens) was observed *in vitro* for MRET-activated nutrient medium in this investigation.

Key words: MRET water, *Staphylococcus aureus*, *Phagocytes*, *Macrophages*, *Neutrophils*, cytotoxic activity, bacteriostatic.

INTRODUCTION

The research was conducted under supervision of Prof. Vladimir I. Vysotskii and Prof. Lydia S. Kholodna, at Division of Microbiology and Immunology, Biological Department of Kyiv National Shevchenko University, Ukraine.

MRET water activator is the stationary source of subtle, low- frequency, resonant electromagnetic field of composite structure. The origin of the low-frequency composite electromagnetic field is the intensive electrical activity of nano-rings formed by linear molecular groups of MRET polymer compound (volumetric fractal geometry matrix) when polymeric body is exposed to the external electromagnetic fields of specific frequency and wavelength [Vysotskii et al., 2005].

The research regarding the physical parameters of water conducted earlier at Moscow State University, Russia confirmed that MRET treatment of distilled water leads to substantial modification of basic physical-molecular properties of distilled water.

The anomalous viscosity of MRET water (subject to very low tangent pressure) and electrodynamic characteristics of MRET water (subject to applied electromagnetic field of low frequency range) confirmed the high level of long-range dynamic structuring of water molecules in polarized-oriented multilayer formations in activated water produced with the help of MRET activation process (Smirnov, 2007b). The prior researches confirmed the ability of MRET water to enhance morphology of blood cells, im-mune response and to inhibit the growth of mutated cells (Smirnov 2006a, b; Vysotskii, 2006).

Taking in consideration the beneficial effect of MRET water on the metabolism of the body and its ability to the inhibition of tumor growth, as well as the high bacteriostatic activity of MRET water confirmed by the previous studies, the goal of this research was to investigate the effect of MRET water on *Staphylococcus aureus* in animal model and *in vitro.*

In the process of the research the effect of MRET activated water was studied in animal mice model on the

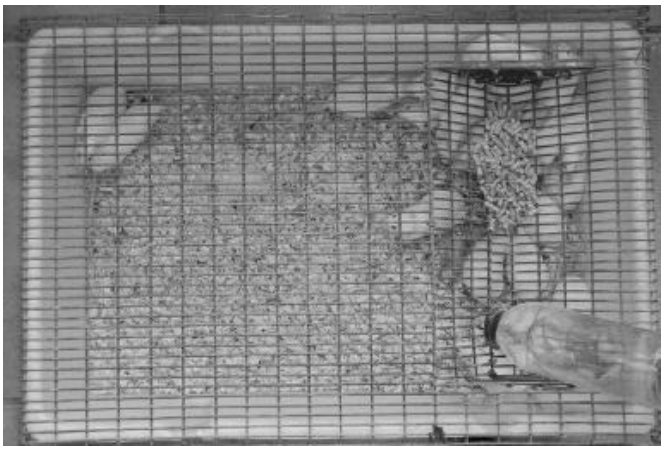


Figure 1. The view of an open-air cage with mice and the container with MRET water available to mice for unlimited consumption (on the right bottom part of the picture).

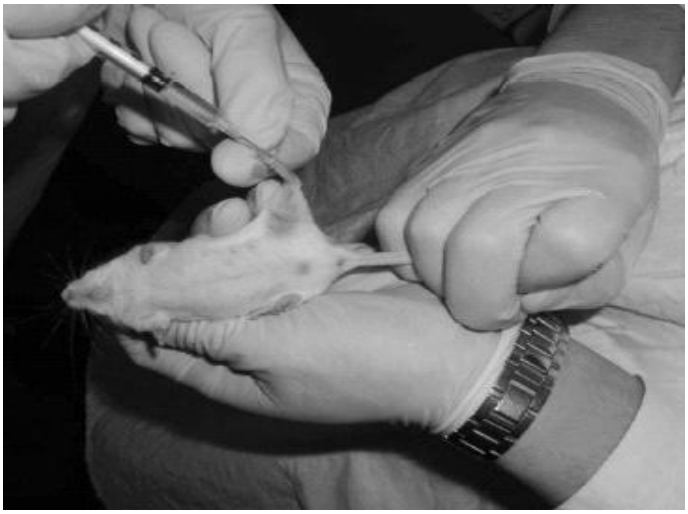


Figure 2. The procedure of inoculation of Staphylococcus aureus culture in the hind left paw of a mouse.

characteristics of weight and cellularity of lymphoid organs of immune system and on functional activity of phagocytes (peritoneal macrophages and neutrophils of the peripheral blood).

MATERIALS AND METHODS

The investigation of the effect of MRET activated water was conducted in two steps: the evaluation of the immune-stimulatory effect following the ingestion of MRET water on the immune-competent cells in the model of mice infected with *S. aureus* Wood-46 (*in vivo*) and the evaluation of the inhibition of growth of culture of *S. aureus* Wood-46 in MRET activated nutrient mediums (*in vitro*). The *S. aureus* Wood-46 culture was received from the Czechoslovak collection of microorganisms.

The research was conducted on 400 mice-male of line BALB in the age of 11 - 13 weeks and of the weight 18 - 21 grams. In the process of investigation all mice were divided into three groups.

Prior to the inoculation of *S. aureus* Wood-46 culture one group of mice consumed MRET water for 4 weeks (Group 1), another group consumed MRET water for 2 weeks (Group 2), the control group consumed non-activated ordinary distilled water. During the following 2 weeks of experiment the first two groups continued to consume MRET water and the control group consumed ordinary distilled water. The first preliminary line of experiments was conducted on 225 mice in order to analyze the persistence of pathogen in the homogenate of kidneys of mice comparing 5 groups of mice (two Group 1 and two Group 2 on 15 and 30 min MRET ac-tivated water and Control). After preliminary experiments the opti- mal 30 minutes MRET activated water (distilled) was chosen for the main line of investigation. The view of an open-air cage with mice is shown on Figure 1. In the process of the investigation two types of staphylococcal infection were studied: the local inflammation and the intraperitoneal infection. In order to induce a local inflammation the culture of *S. aureus* Wood-46 was inoculated in the hind left paws of mice (Figure 2). For other series of experiments the inoculation of culture of *S. aureus* Wood-46 was conducted intra-peritoneal in dose LD30 in order to spread the infection all over the body.

The second step of investigations was conducted *in vitro* based on the analysis of the growth of staphylococcal culture on meat-peptone agar (MPA) at a temperature of 37°C during 18 - 24 h with different initial concentrations of *S. aureus* (from 10^1 - 10^9 bacteria/ml). The samples were treated with the help of MRET activator during different periods of time (in the range of 15 - 60 min) right after the introduction of staphylococcal culture to MPA.

The following experiments were designed to study the effect of MRET activation on the process of growth and development of *S. aureus* Wood-46 culture *in vitro* in nutrient medium (MPA). The bacterial culture was grown overnight to stationary phase and then platted on MPA in the form of suspension at different inoculation densities. The MPA with culture was MRET activated during the different periods of time (activation for 15, 30, 45 and 60 min respectively) following the requirements of sterility. Petri dishes with activated and non-activated medium (MPA with culture) were covered with glass caps (aerobic environment) and placed in the thermostat for cultivation at a temperature of 37° during 18 - 24 h.

After the cultivation the morphological and tinctorial properties of cultures were observed and the numbers of colonies grown on MPA were counted. The bacteriostatic activity of MRET activated nutrient medium (MPA) was measured as an Index of Bacteriostatic Activity (IBA). An Index of Bacteriostatic Activity is defined as a coefficient of the inhibition of growth and reproduction of pathogens in bacteriostatic medium, particularly in MRET activated nutrient medium. It is calculated as reduction of the number of colonies (CFU – Colony Forming Units) in MRET activated medium related to the control samples not exposed to the activation:

$$IBA = (N_{control} - N_{act})/N_{control}$$

Where N – number of bacteria colonies (CFU) in Control (non-activated) and MRET activated nutrient medium, respectively.

In order to verify the sterility of experiments Petri dishes with nutrient medium (MPA) without staphylococcal culture were exposed to the process of activation and then were kept in the thermostat. No colonies of culture were observed that confirms the sterility of environment. (Table 1)

RESULTS

The effect of MRET water on staphylococcal infection *in vivo* in animal model

The significant protective properties of MRET water were confirmed by substantial decrease of *Staphylococcus*

Table 1. The effect of consumption of MRET water on the persistence of pathogens of staphylococcal infection in homogenate of kidneys of mice.

Groups of experimental animals	Period of activation, minutes	Number of CFU in 1 ml of homogenate of kidneys in 1 day	Number of CFU in 1 ml of homogenate of kidneys in 3 days	Number of CFU in 1 ml of homogenate of kidneys in 5 days
Group 1, N = 15	15	24266 ± 1330*	43227 ± 5600*	15160 ± 1310*
Group 1, N = 15	30	19316 ± 1460	29600 ± 1890*	14000 ± 1660*
Group 2, N = 15	15	23387 ± 2760*	42550 ± 4500*	14550 ± 1750*
Group 2, N = 15	30	24060 ± 870*	41760 ± 3090*	10600 ± 1200*
Control, N = 15	–	19000 ± 2620	76590 ± 4340	31250 ± 2220

CFU – colony forming units
– marks statistically significant results with < 0.05 compared to Control.

CFU (colony forming units) in homogenate of kidneys of mice on MRET water compared to control group of mice following the intra-peritoneal staphylococcal infection after the first 24 h. For this purpose the kidneys of animals were dealt individually. The analysis of data in the beginning of experiments leads to the conclusion that significant decrease of pathogen's colonies in homogenate of kidneys of mice on MRET water begins only after 24 h following the inoculation of *S. aureus* . The results on 30 min activated water were much better than on 15 min activated water and all further experiments were conducted on 30 min activated water.

The consumption of MRET water reduced the death rate from 30% (control group) to 0% (MRET groups) during the first 9 days of experiment. There was no case of animal death in all investigated groups within the first 24 h after intra-peritoneal inoculation of *Staphylococcus* culture, which is a pretty standard result. During the next 8 days 30% of animals died in control group which is an expected result for such experimental procedure. There was no death case in both groups of mice that ingested MRET activated water and it is a very unusual result. Nevertheless, the main consequences of *Staphylococcus* infection do not manifest in death of animals as in case of oncology diseases. *Staphylococcus* bacteria affect the live systems and organs of the body. These pathogenic microorganisms cause inflammations, suppurations, abscesses, furuncles, quinsy, cepsical conditions, etc. That's why a detailed investigation of the process of stimulation by MRET water of phagocytes and of lymphoid organs of immune system of mice infected with *S. aureus* culture was conducted and is presented in this report.

The development of the local acute inflammation is essentially suppressed in case of ingestion of MRET activated water

The local inflammation was induced with the help of the inoculation of *S. aureus* culture into the hind left paw. The ordinary inflammatory reaction was observed in the group of mice on non-activated water: the intensive reddening of the hind left paw (Figure 3). Both groups of mice on MRET water did not develop any reddening of the hind left paw inoculated with *S. aureus* culture (Figure 4) . The results of this experiment confirm the fact of the substantial inhibition of inflammatory infection in case of the regular consumption of MRET water.

The consumption of MRET water stimulates the activity of phagocytic system and the level of natural resistance of animals to pathogenic microorganisms.

For the following series of experiments the inoculation of *S. aureus* Wood-46 was conducted intra-peritoneal in dose LD30 in order to spread infection all over the body.

The phagocytic system is one of the main factors of natural non-specific cellular resistance to infections and inflammations. It is the first line of protection of an organism against penetration and reproduction of pathogenic microorganisms. The protective role of phagocytic cells is based on their capacity to identify, engulf and utilize the alien agents penetrated into internal environment of a macro- organism. Phagocytosis is the main mechanism of natural resistance especially at the first stage of contagious process; it is a regular part of formation of the specific immune response.

The most common methodology applied in the studies of the functional activity of phagocytes is the examination of their phagocytic (engulfing of alien cells) and oxygen-dependent bactericidal activity. Phagocytic activity of neutrophils and macrophages is estimated based on Phagocytic Index (percentage of phagocytes which engulfed test-bacteria) and on Phagocytic Number (average number of test-bacteria engulfed by one phagocyte). The cultures of *S. aureus* and Latex are usually used as test-bacteria. The oxygen-dependent bactericidal activity of phagocytes is studied with the help of NBT-test: an oxygen-dependent reduction of Nitro Blue Tetrazolium into an insoluble Diformazan of Nitro Blue Tetrazolium derivative by phagocytes. With the help of NBT-test it is possible to distinguish the activated phagocytes from the non-activated ones.

MRET water stimulated the phagocytic capacities of neutrophils of a peripheral blood and peritoneal macro-

Figure 3. The view of paws of a mouse on non-activated water (reddening of the injected paw) in 24 h after the injection of Staphylococcus culture.

Figure 4. The view of paws of a mouse on MRET activated water (no reddening of the injected paw) in 24 h after the injection of Staphylococcus culture.

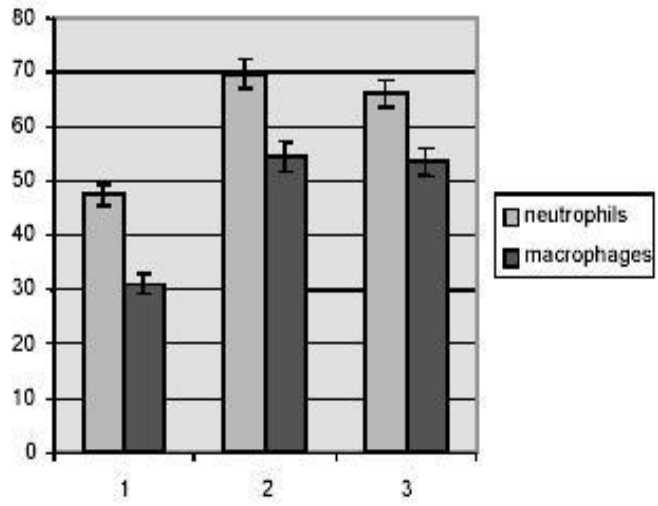


Figure 5. Phagocytic Index of neutrophils and macrophages in two weeks of experiment (object of phagocytosis - Staphylococcus aureus): 1 – Control group; 2 – Mice on MRET water (preventive for 4 weeks); 3 – Mice on MRET water (preventive for 2 weeks).

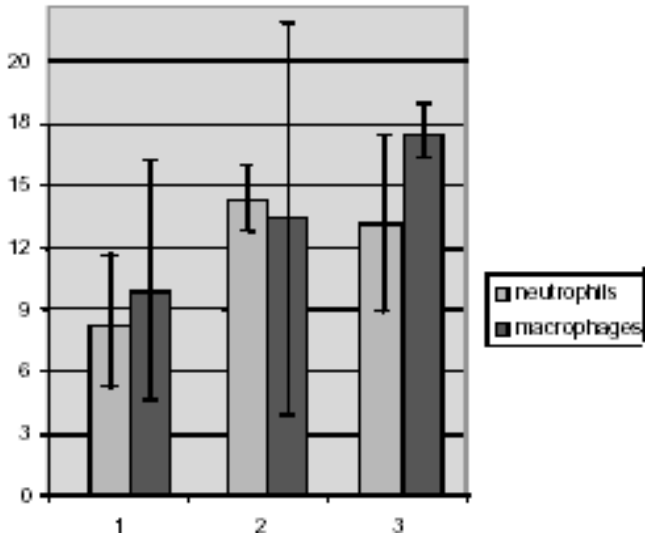


Figure 6. Phagocytic Number of neutrophils and macrophags in two weeks of experiment (object of phagocytosis – Staphylococcus aureus): 1 – Control group; 2 – Mice on MRET water (preventive for 4 weeks); 3 – Mice on MRET water (preventive for 2 weeks).

phages increasing their phagocytic activity, particularly Phagocytic Index (Figure 5) and Phagocytic Number (Figure 6). It also stimulated their oxygen-dependent bactericidal activity, particularly the increase of quantity of NBT-positive phagocytes (Figure 7).

These experiments confirmed the increase of effective potentials of phagocytes, which constitute one of the main factors of natural protection of an organism and initiate the immune response. The analysis of data in the beginning of experiments leads to the conclusion that significant intensification of phagocytic and bactericidal activity of macrophages and neutrophils of mice on MRET water begins only after 24 h following the intra-peritoneal inoculation of *Staphylococcus* culture. At the end of two weeks of experiment the mean values of studied parameters in both groups of mice on MRET water substantially increased compared to the control group. The differences in mean values of the parameters of functional activity of phagocytes of groups of mice consuming MRET water compared to the control group of mice on non-activated water were statistically significant with $p < 0.05$ (for Phagocytic Index and NBT- test). These results confirm the significant intensification of phagocytic and bactericidal activity and of immune system response following the

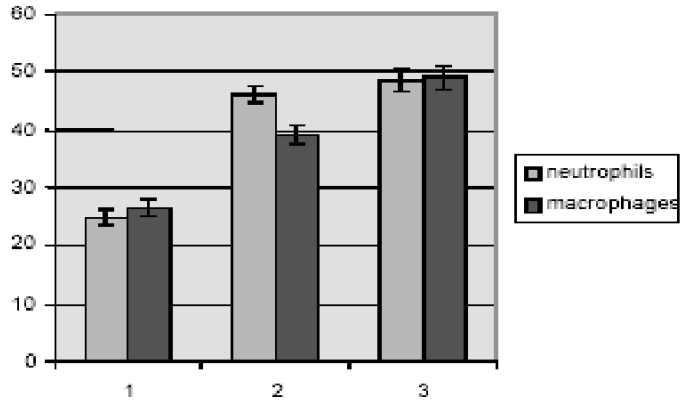


Figure 7. Oxygen-depended bactericidal activity (NBT-test) of neutrophils and macrophages in two weeks of experiment: 1 – Control group; 2 – Mice on MRET water (preventive for 4 weeks); 3 – Mice on MRET water (preventive for 2 weeks).

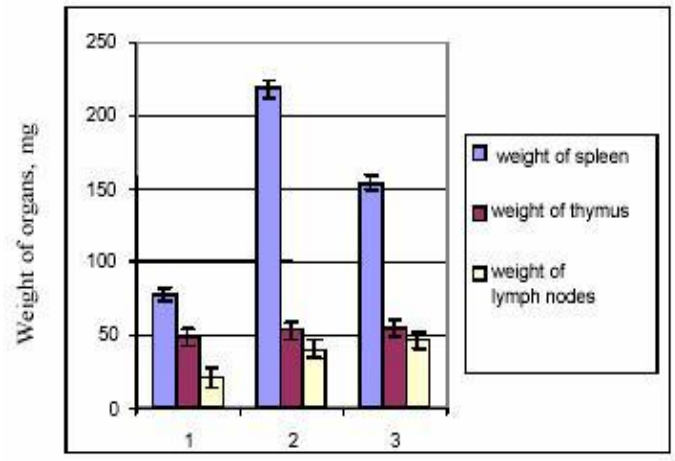


Figure 8. The weight of lymphoid organs in two weeks of experiment: 1 – Control group; 2 – Mice on MRET water (preventive for 4 weeks); 3 – Mice on MRET water (preventive for 2 weeks).

consumption of MRET water.

The differences in mean values of studied parameters for the groups of mice on MRET water compared to each other were statistically insignificant, which confirms the similarity of the level of beneficial effect of MRET water in both groups. This fact also confirms that the regular consumption of MRET water provides health benefits in rather short time (2 weeks in case of the animal mice model).

The consumption of MRET water substantially enhances the immune activity of lymphoid organs

By the end of another series of experiments in both groups of mice on MRET water was observed substantial

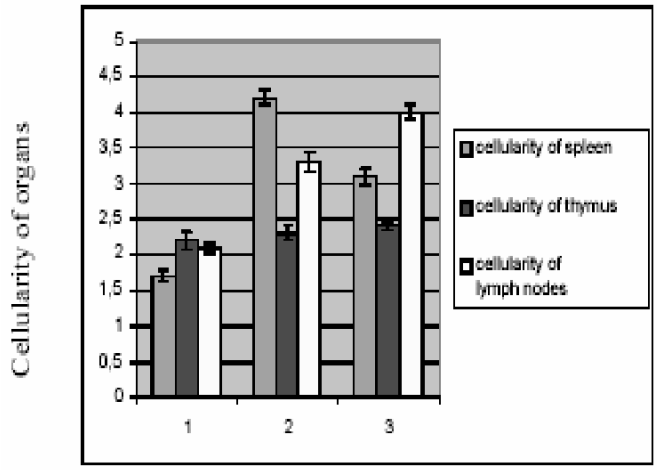


Figure 9. The cellularity of lymphoid organs in two weeks of experiment: 1 – Control group; 2 – Mice on MRET water (preventive for 4 weeks); 3 – Mice on MRET water (preventive for 2 weeks).

statistically significant ($p<0.05$) increase in the weight and the cellularity (quantity of cells) of the spleen and lymph nodes as well as an insignificant increase in the weight and cellularity of the thymus (Figures 8 and 9).

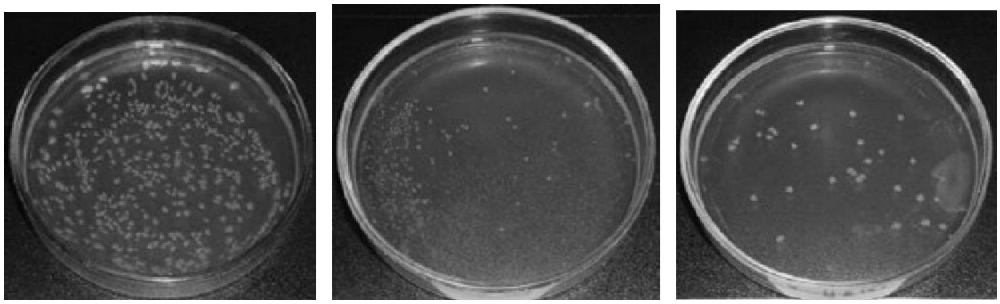
These results confirm the fact of significant intensification of immune system response in animals on MRET water subject to *Staphylococcus* infection. The difference in studied parameters between the groups of mice on MRET water (4 and 2 weeks of preventive consumption of MRET water) was insignificant which confirms quite fast beneficial effect of MRET water on the immune activity of lymphoid organs.

In the beginning of experiment the cellularity and the weight of lymphoid organs in MRET groups did not show the distinct tendency to modifications. It is reasonable to admit that the consumption of MRET water affects the weight and the cellularity of lymphoid organs only during the infection period.

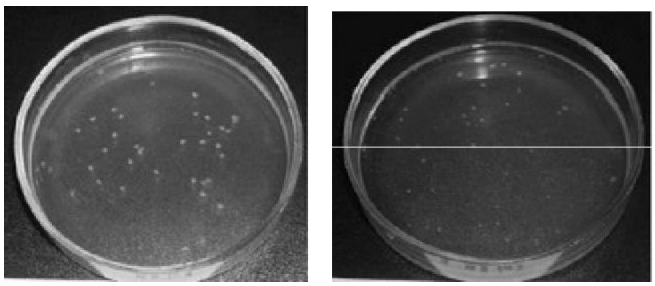
The effect of MRET activation on the process of *staphylococcal* culture growth in nutrient medium

Following the investigation the direct correlation between times of activation (t_{act}), initial concentrations of *staphylococcal* culture (N_0) and a number of colonies grown on MRET activated medium were observed. The results are presented below in the form of a series of photos of Petri dishes with the colonies grown on MPA surfaces and the following diagrams based on the data of these experiments (Figure 10 - 15).

In the process of investigation the effect of MRET activation on the growth of staphylococcal culture at rather small initial concentration of pathogens was analyzed. The data corresponding to higher initial concentrations $N_0 > 10^3$ bacteria/ml were not analyzed due to the difficulties related to calculation of very high values of a number of colonies, despite the fact of the high bacteriostatic activity



$N_0 = 10^3$ bacteria/ml, Control t_{act} = 15 min t_{act} = 30 min



t_{act} = 45 min t_{act} = 60 min

Figure 10. The effect of time duration of MRET activation on the inhibition of growth of culture of *Staphylococcus aureus* Wood-46 with initial concentration of culture $N_0 = 10^3$ bacteria/ml.

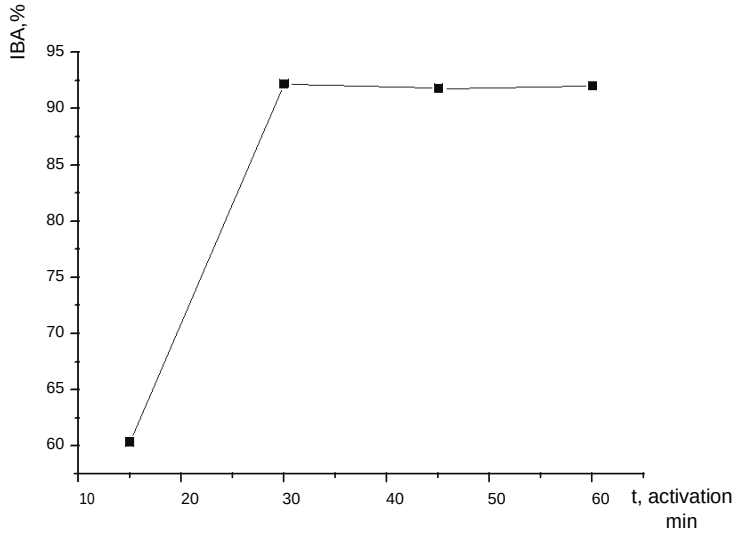
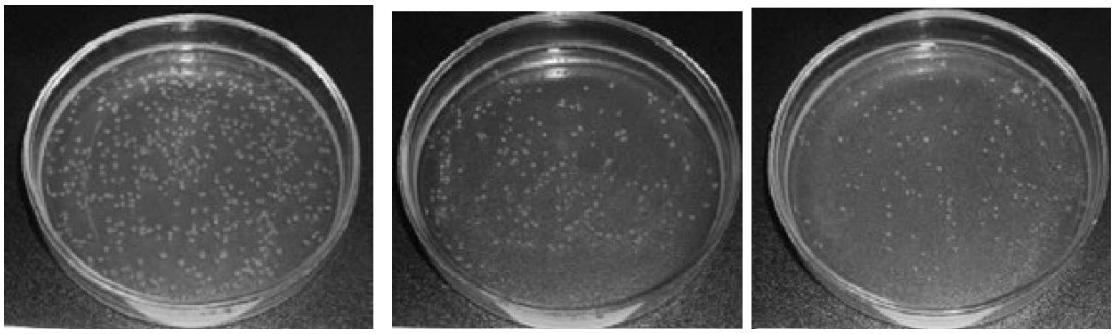


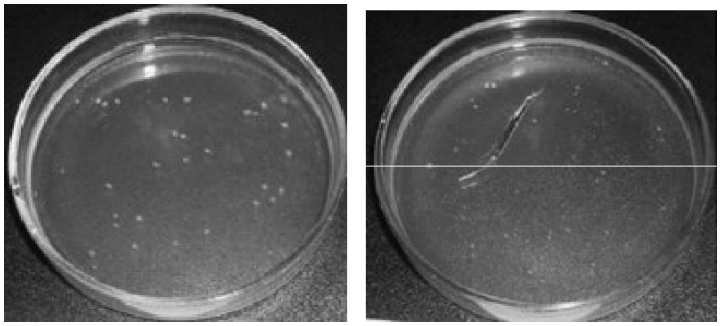
Figure 11. The effect of time duration of MRET activation on the inhibition of growth of culture of Staphylococcus aureus Wood-46 with initial concentration of culture $N_0 = 10^3$ bacteria/ml. IBA – Index of Bacteriostatic Activity (reduction of the number of colonies related to the control samples not exposed to activation).

of MRET activated nutrient medium in case of high initial concentrations.

The highly significant bacteriostatic effect of 92 - 93% was observed after MRET activation for 30 min and more of cultures with initial concentration $N_0 = 10^3$ bacteria/ml (Figures 10 and 11) and of 70 - 90% with initial concentration of $N_0 = 10^2$ bacteria/ml (Figures 12 and 13). In case of cultures with low initial concentration $N_0 = 10^1$ bacteria/ml the bacteriostatic activity in 15 min activated nutrient medium exceeded 93% and in 30 min activated



$N_0 = 10^2$ bacteria/ml, Control t_{act} = 15 min t_{act} = 30 min



t_{act} = 45 min t_{act} = 60 min

Figure 12. The effect of time duration of MRET activation on the inhibition of growth of culture of *Staphylococcus aureus* Wood-46 with initial concentration of culture $N_0 = 10^2$ bacteria/ml.

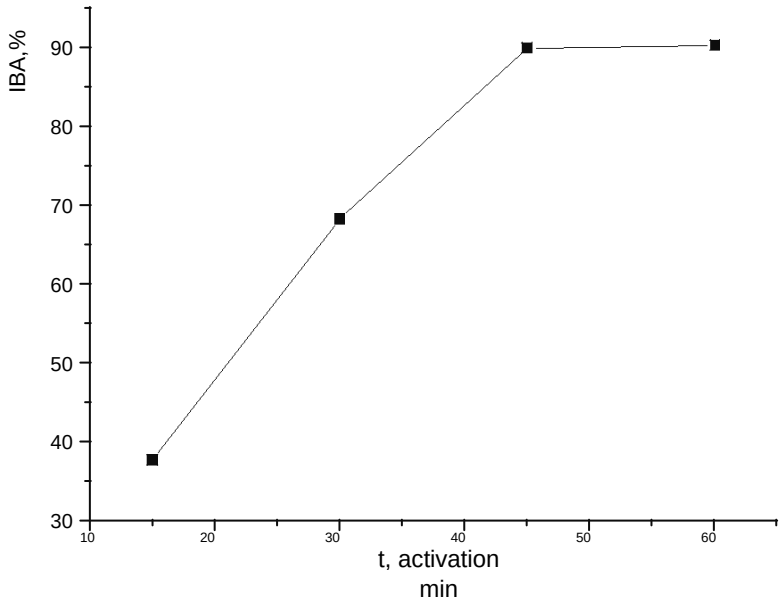
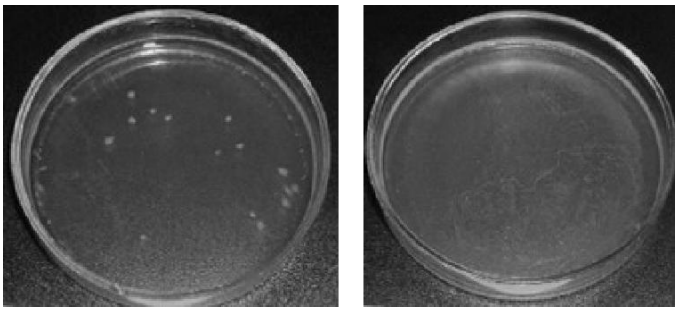


Figure 13. The effect of time duration of MRET activation on the inhibition of growth of culture of Staphylococcus aureus Wood-46 with initial concentration of culture $N_0 = 10^2$ bacteria/ml. IBA – Index of Bacteriostatic Activity (reduction of the number of colonies related to the control samples not exposed to activation).



N_0 = 10 bacteria/ml, Control t_{act} = 15 min

Figure 14. The effect of time duration of MRET activation on the inhibition of growth of culture of Staphylococcus aureus Wood-46 with initial concentration of culture $N_0 = 10^1$ bacteria/ml.

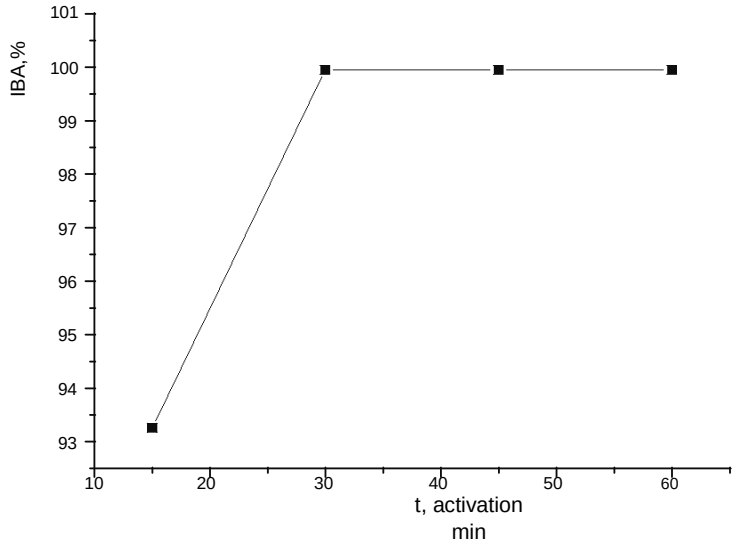


Figure 15. The effect of time duration of MRET activation on the inhibition of growth of culture of *Staphylococcus aureus* Wood-46 with initial concentration of culture $N_0 = 10^1$ bacteria/ml. IBA – Index of Bacteriostatic Activity (reduction of the number of colonies related to the control samples not exposed to activation).

nutrient medium was observed 100% inhibition of *staphylococcal* colonies (Figures 14 and 15).

Conclusions

The consumption of MRET activated water significantly enhances the factors of natural resistance of the body which constitute the first line of protection of an organism against the penetration and reproduction of pathogenic microorganisms.

The analysis of data in the beginning of experiment leads to the conclusion that significant changes in all studied parameters of mice on MRET water (decrease of pathogen colonies in homogenate of kidneys, increase of the weight and the cellularity of lymphoid organs, intensification of the phagocytic and bactericidal activity of macrophages and neutraphils) begins only after 24 h following the inoculation of *Staphylococcus* culture. In other words the consumption of MRET water increases the potentials of immune capacities of the body to counteract the infections without any changes in the vital parameters of immune organs and functions prior to the penetration of infectious pathogens in the body.

At the end of two weeks of *in vivo* experiment the mean values of studied parameters in both groups of mice on MRET water (preventive for 4 and 2 weeks respectively) significantly increased compared to the control group. The differences in mean values of the studied parameters of the groups of mice consuming MRET water compared to the control group of mice on non-activated water were statistically significant with $p < 0.05$ (for most of the para-

meters). Particularly, the consumption of MRET water reduced the death rate from 30% (control group of mice on non-activated water) to 0% (two groups of mice on MRET water) during the first 9 days of experiment after intraperitoneal inoculation of *Staphylococcus* culture. The significant bacteriostatic effect of 70 - 100% (depending on initial concentrations of pathogens) was observed *in vitro* for MRET-activated nutrient medium in this investigation. These results confirm the significant intensification of phagocytic activity and of immune system response following the consumption of MRET water. The differences in mean values of studied parameters for the groups of mice on MRET water compared to each other were statistically insignificant, which confirms the similarity of the level of the beneficial effect of MRET water in both groups. This fact also confirms that the regular consumption of MRET water provides health benefits in rather short period of time (2 weeks in case of the animal mice model).

The results of *in vitro* investigation provide the evidence regarding the high efficacy of MRET activation on the inhibition of growth of colonies and reproduction of *staphylococcal* microorganisms and thus confirm high bacteriostatic (antibacterial) activity of MRET water.

MRET activation of the water based nutrient medium with suspended staphylococcal culture leads to the origination of high bacteriostatic activity of nutrient medium which depends on the time duration of activation and initial concentration of culture cells. The bacteriostatic activity increases following the increase of time of activation (the times of activation up to 60 minutes were studied). The efficacy of bacteriostatic activity increases following the decrease of initial concentration of the suspension of *staphylococcal* culture. The process of MRET activation is most effective on culture suspensions with the concentration not more than 10^3 bacteria/ml.

The results of investigation provide the evidence regarding the high efficacy of MRET activation on the inhibition of growth of colonies and reproduction of staphylococcal microorganisms *in vitro.* These results allow admitting that the process of MRET activation and the sterilization effect of MRET water can be applied in food industry and for water purification.

REFERENCES

Smirnov IV (2007a). “MRET Activated Water and its Successful Application for Preventive Treatment and Enhanced Tumor Resistance in Oncology” http://www.eurojour nals. com/Vol%2016%20No%204.htm Eur. J. Sci. Res. 16(4): 575-583.

Smirnov IV (2007b). “The Anomalous Low Viscosity and Polarized-Oriented Multilayer Molecular Structure of MRET Activated Water” Explore 16(4): 37-39.

Smirnov IV (2006a). “The Physiological Effect of MRET Activated Water on Patients Suffering from AIDS” Explore. 15(2): 37-40

Smirnov IV, Peerayot T (2006b). “The Physiological Effect of MRET Activated Water” Explore 15(1): 38-44.

Vysotskii VI (2006). “The Biophysical Model and Experimental Observation of Strong Inhibition Activity of Water Activated with the help of MRET Process” Program and Abstract Book, International Congress on Medical Physics and Biomedical Engineering, Seoul, Korea.

Vysotskii VI, Smirnov IV, Kornilova AA (2005). “Introduction to the Biophysics of Activated Water” Universal Publishers.