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Editorial

Constantin Munteanu

The editorial of this number is reserved for a paradigm presentation of Balneology and how a researcher in molecular and cellular biology adapts to the thematic content and experimental area.

The balneary future turisms will be successful in Romania if succeed in transforming the tremendous potential of natural healing factors with proposed strategies in a modern health and welfare resorts center.

They will have complex products, offer balnear and recovery therapeutic treatment and for welfare and health turism, focused on life quality and closely intertwined with the maintenance of health. These resorts can become reference centers for people’s welfare needs.

To answer of balneary tourism market requirements is necessary to create polyanvalent resorts by broadening and diversifying the resorts basic profile parallel with development of new profile: stress removal, rehabilitation, beauty, thalassotherapy, prophylaxis.

Among the general issues required to build modern balneoturistic resorts include:

- thorough analysis to establish the register of natural healing factors, useful mineral reserves and the level of use;
- setting the optimal profile and specialization of the resorts as a basis for modernization and/ or creation the welfare centers;
- outlining the best solutions for functional zoning and general and specific items of infrastructure;

A strategic recovery of romanian balneary tourism potential will allow to repositioning on national and international market.

The success of this action depends on the involvement of policy makers at micro and macro economic level and the social effects for our country can be significant.

The role of balneary resorts in a modern society is today made in light of changes that human civilisation faces. The rhythm of life, the daily stress, the avalanche of information, the „consumer- devouring” state, force us to find moments for relaxation and rest, treatments, recreation and leisure. This bring us to want to „change the air”.

We all want to go in vacation in the mountains or the sea, in the mountain circuit or balneary resort. We are looking the rest and strengthening the body, we want to „recharge the batteries” and find the solution for our pain through methods that came from nature.

Tourism, treatment and recreation in a natural environment, other than home and work, in winter or summer became o constant concern to us all. This concern for rest, recreation and treatments, generated by human needs, create premises for the development of the climatic and balneo-climatic resort network.

Sustainable development of balneary resorts should be linked with inventory and study of therapeutic effects and a efficient use of resources and natural and therapeutic factors. Unique balneary resources such as hot springs, mineral springs, mud, molettes and bioclimate, are successfully used in balneary area.

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Sustainable development must take in consideration the human resource. If the specific human resource of accommodation facilities and balneary treatment, respectively medical staff, are well know and integrated into the resort structure, the human resource responsible for indentifying and researching natural therapeutic factors is less know.

However nature has the right answer, directly and firmly. As the climat change is the natural response to excessive consumerism and devouring, in the same measure degradation and inefficient action of natural factors cand be installed in the resort life if the role of research is forgotten.

Modern means and experimental technique of current research has new valences regards to the study of natural therapeutic factors. The biologist, concerned by the all aspects of life phenomena is today more concern to find the answer to the question „how the natural factors action on human body?; which are the cellular and molecular springs involved?”.

We taking account that how the body reacts to excitatory factors is caused by the nature of disease and her biological spring.

The clinical scientific research conducted by balneology doctor represents an important pillar of the balneary research. This pillar can be accompanied to another: scientific experimental research in biology, thus integrating the data into a unitary system for understanding the role of natural therapeutic factors on the body.
The IX Agenda of National Conference of Balneology

The conference THEME: natural therapeutic factors - their role in promoting human health and survival of balneology resorts

Organizers:
National Institute of Rehabilitation, Physical Medicine and Balneoclimatology
Romanian Balneology Association

Locatie: Doina Complex, Neptun

Theme:
- Marathon of balneary resorts- Features of the medical resort business and the results of research activities (for this section, please register in time, limited to 12 places and get the touch with organizers, for presentation details)
- Studies and researches in the filed of therapeutic mud
- Sulphurous waters- mechanism of action and therapeutic effects.
- Studies on micro and bioclimat.
- Medical recovery in balneary resort.

Preliminary program

Day 1
12 may 2011
14.00 – 19.00 Registration of participants
19.00 Official opening
20.00 Cocktail

Day II
13 may 2011
9.00 – 11.00 Marathon of balneary resorts (I)- Features of the medical resort business and the results of research activities
Coffee Break
11.30 – 13.30 Marathon of balneary resorts (II)- Features of the medical resort business and the results of research activities
Coffee Break
15.00 – 17.00 Studies and researches in the filed of therapeutic sludge
Sulphurous waters- mechanism of action and therapeutic effects.
20.00 Festive Dinner

Day III
14 mai 2011
9.00 – 11.00 Studies on micro and bioclimat
Break
11.30 – 13.30 Medical recovery in balneary resort (I)
Work closing
Explore the potential speleotherapeutic through molecular and cell biology techniques

Munteanu Constantin¹, Simionca Iuri³, Munteanu Diana², Hoteteu Mihai³

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² Romanian Balneology Association
³ National Institute of Rehabilitation, Physical Medicine and Balneoclimatology

Abstract

Objective: Exploring the speleotherapy effects on morphology and physiology of dermal and pulmonary fibroblast obtained from Wistar rats tissue in normal conditions and after induction of experimental “astma” awareness with ovalbumin.

Materials and methods: Before initiation of dermal and pulmonary fibroblast cultures, 60 of Wistar rats 75-100 g were divided into two groups: control and sensitized with ovalalbumin. 10 animals of each group were sent to Cacica and Dej salt mines and maintained in a speleotherapy regime. Another 10 animals in each group were monitored separately in INRMFB Biobase. Dermal and pulmonary fibroblast cultures were initiated by enzymatic techniques from appropriate tissue taken of each group Wistar rats. Morphological monitoring was done by phase contrast microscopy; biochemical and molecular changes of cultures obtained from animals treated speleothropic compared to control, was experimental established by electrophoresis and Western Blotting techniques.

Results: Experimental data revealed the expression of several proteins after the speleotherapeutic treatment. These data were analysed compared with control, using a specific software.

Conclusions: Speleotherapeutic treatment of Wistar rats caused significant differences in morphology and protein expression of dermal and pulmonary fibroblast grown in the laboratory. These differences support the protective effects of speleotherapy compared with data obtained from animals untreated and sensitized with ovalbumin, having induced experimental asthma status.
Introduction

Speleotherapy uses specific conditions of caves and salt mines to treat many diseases, particularly respiratory type. The air of salt mines are poorer in dust; this could form the basis of allergic reactions or asthma attacks. This reduces any irritation and thus the disease symptoms are reduced or even completely removed during the stay of patient in salt mine. But this aspect can’t explain the speleotherapy effect to long time.

The treatment of the asthma involves the patient stay in the underground for 2-3 hours/ day, for 2-3 months. An earlier study describes a speleotherapeutic regime consisting in 4 hour / day for 6-8 weeks, for a 100 patient with chronic obstructive pulmonar and diseases. The study results showed a health improvement of patient that lasted from 6 to 7 years (Skulimowski, 1965).

Asthma is a chronic disease characterized by airways inflammation who became hyper-responsive and thus changes their architecture, a process called remodeling. The parenchymal cells, including epithelial, mesenchymal and endothelial cells are responsible for the structure of the lung. Recent studies have suggest that the function of the epithelial cells, smooth muscle cells and fibroblast in culture, obtained from the lungs of people with asthma differs from similarly grown cells from healthy people. These functional differences related to repair and remodeling processes could contribute to structural change of airway (Sugiura et al., 2007).

The current study was designed to investigate the microclimate influence of Cacica and Dej salt mines on cellular morphology and electrophoretic expression of pulmonary fibroblast in vitro, obtained from Wistar rats in normal and awareness (with ovalbumin- „asthmatic”) conditions.

Fibroblasts were grown from parenchyma of control rats, sensitized with ovalbumin, untreated and treated in salt mine after the sensitization with ovalbumin- in speleotherapy cure. The fibroblast form culture may vary depending on the substrate that are grown and the space for movement.

Use of pulmonary fibroblast culture to verify therapeutic properties of the salt mine microclimate by speleotherapeutic cure is a scientific method to determine prevention, treatment and recovery medical methodology of patient with various pulmonary problems.

Experimental methodology

By in vitro studies can notice: the cell morphology, protein synthesis, secretion of certain substances, cell metabolism, cells interaction through receptors and ligands, capture or release of electrolytes and other substances reaching in cell.

To observe and characterization in vitro cells response, subject to speleotherapeutic action will use cell cultures obtained by specific process from Cell Culture Laboratory.

Essential protocols for cell culture are represented by: obtaining primary cultures, subculturing cells, trypsinisation, cell cultures crushing, cell counting using hemocytometer, cell viability assessment: tripan bule exclusion method, neutral red staining; cytotoxicity tests: lacticdehydrogenase study, MTT test, cell growth curve determination, senescente cells histochemical evidence using β galactosidase, performance of cloning determination, thawing and cryopreservation cells.

Evaluation of cellular and molecular changes can be done by optical microscopy, who revealed: the cellular morphology, cell viability studies, immunohistochemistry studies, proteomic studies made by specific techniques, including electrophoresis and Western Blotting, basic biochemical parameters determination in culture, cell physiology studies, studies on cellular senescence, cell signaling studies.

Protein electrophoresis of cell homogenous total lised is intended to setting changes occurred in proteins expression of dermal cultures derived from rats subjected to speleotherapeutic treatment.

Protein electrophoresis in polyacrylamide gel was done under distortive conditions according to the techniques describe by Laemmli (1979). The cultures was washed with TFS, scrape from culture plate and lysates in buffer containing 0,5 M Tris-HCl, pH 6,8 + 0,05% BPB + glycerol 10% + 10% SDS.

Detection through Western Blotting is performed by an indirect method in which the secondary antibody is coupled to peroxidase.

Densiometric analysis for assess the relative amount of protein was done with SCIE-PLAS VISION using the gel analysis software from SYNGENE, version 4.00.
Results

Control dermal cell culture of 7 days has a heterogeneous cellular composition composed by dermal fibroblast and keratinocyte epithelial cells. After 7 days from cultivation reach an advanced preconfluence level.

Dermal cell culture of 7 days from sensitized animals of 14 days with ovalbumin has a different cellular composition from control culture being composed by fewer dermal fibroblast and more keratinocyte epithelial cells. After 7 days from cultivation reach an lower level than control.

Dermal cell culture of 7 days from sensitized animals of 14 days with ovalbumin and subsequently exposed for 14 days in Cacica salt mine, has a slightly cellular composition from control being composed by fewer dermal fibroblast and more keratinocyte epithelial cells.

The ration of the two cell types is intermediate between control culture and that of sensitized group of animals. The epithelial cells vacuolation is more pronounced in control case, while fibroblast may acquire many morphopathological characteristics. Dermal cell culture of 7 days from sensitized animals for 14 days with ovalbumin and subsequently exposed for 14 days in Cacica salt mine, has a slightly cellular composition from control being composed by fewer dermal fibroblast and more keratinocyte epithelial cells.

The ration of the two cell types is intermediate between control culture and that of sensitized group of animals, very similar with Cacica case.

The control pulmonary fibroblasts culture of 9 days has a more homogenous cellular composition from dermal cells culture consisting only fibroblasts. After 9 days of cultivation reach an advanced preconfluence level. Cell division has a high frequency. The control cell morphology is similar to literature.

The control pulmonary fibroblasts culture of 9 days from sensitized animals for 14 days with ovalbumin changing substantially from control culture by number of cell reduction, frequency of cells reduction and increased of morphopathological fibroblasts characteristics.

Fibroblasts cell culture of 9 days from sensitized animals for 14 days with ovalbumin and subsequently exposed for 14 days in Cacica salt mine, showed an improvement of morphological parameters cells compare with ovalbumin sensitized animals.

Microscopically can see an increase in number cells without achieved cell density of control.

Conclusions

- Microscopic morphological analysis culture reveal cell regeneration after exposure in Dej and Cacica salt mines, compare to culture obtained from ovalbumin sensitized animals.
- Cell morphology observations are confirmed by electrophoretic analysis which demonstrates that the exposure in Cacica and Dej salt mines favors dermal cells and pulmonary fibroblasts in vitro through the changing profile of several proteins and total amount of protein determination.

Bibliography

Experimental design for speleotherapeutic exploration potential.
Bronchial asthma affects up to 10% of the developed countries population, its prevalence increasing in all world [Lemanske si Busse, 2003]. Therapy with bronchodilators, corticosteroids, leukotriene inhibitors, mastoid cells stabilizers and recent with IgE receptor antagonists have been shown an improvement of asthma symptoms.

To solve the existing problems in allergy, pulmonology and medical recovery field and for use of natural therapeutic factors in patient treatment with different pathologies international scientific community appealed to specialists, medical, ecological and social programs.

The new scientific and practical directions in therapy of the most severe allergic diseases-bronchial asthma use underground medium of salt mines and caves. These therapy method was name speleotherapy- from greece „spelaion”- cave, gap and „therapy”- treatment. Today the speleotherapy is recognized as therapy in underground of salt mines and caves with natural theraoeutic factors for many deseases (Iu.Simionca și al.,2005, 2008).

Speleotherapy presents an great scientific interest and is a future direction in heath and environmental area.

One of the perspective salt mines use in medical and balneoclimatic tourism purpose from Romania is Turda Salt Mine.

Turda Salt Mine is one of the historical monuments of Romania, from Cluj and a touristic attraction at national level especially for Bai Sarate Turda, Durgau salted lakes and the ruins of Potaissa roman castrum where was stationed the Vth Macedonica Legion about 2000 years ago.

The exploitation of salt from Turda in current microdepression of Baile Sarate has a special interest during the roman occupation in Dacia. The first documentary of mine attestation dating from XII century when avid rocks, minerals and fossils collector- Joanne Fridvaldscky says- „is so famous that has no equal in all eastern”.

With Saraturile Turzii was declared natural reserve with national interest and became a historical museum of salt mining.
At the request of S.C. „Turda Salt Mine Durgau” S.A., under a service contract for environmental quality evaluation and presence of therapeutic underground factors of Turda Salt Mine, National Institute of Rehabilitation, Physical Medicine and Balneoclimatology has decided to make an experimental study „Medico-biological complex study of the Turda underground on laboratory animals with induced pathology in order to evaluate the speleotherapeutic factors and the possibilities of salt mine underground use in health and balneary tourism”; following activities are planned:

- Multidisciplinary study of the underground mines is different location/ cavities of Turda Salt Mine including microbiological investigations of air (concentration of organisms, species identification) at 30 cm, 70 cm and 1 m 50 cm from the "salt soil"; a portion of the wall surface and "salt soil", the concentration of gases - CO\textsubscript{2} and other 7-9 gases, including those classified as pollutants, evaluation of aerosol concentration and dispersion in salt mines at different levels, the concentration of positive and negative air ions of underground; investigation microclimatic - temperature, humidity, air flow speed, atmospheric pressure, the determination of underground air salinity, investigation of radioactivity - values radiation dose β / γ radiation, radionuclide concentration in the layer ("wall") salt concentration of radon in the air underground, surface investigations witness.

- Achievement of experimentally induced disease model (with of antigen sensitization/ experimental bronchial asthma) in a group of Wistar rats that will be use later in an experimental study of the effect of Turda Salt Mine speleotherapy cure.

- Realisation of schemes and specific types of speleotherapeutic treatment cure. Obtained data on the environmental study and analysis of underground status Turda Salt Mine microclimatic physical, chemical, biological and microbiological factors, which represents the average underground salt mine and subsequent implementation of the experimental model of pathology induced on laboratory animals (Wistar Rats - WR) for the experiment on evaluating the potential therapeutic effect of the underground salt mine respectively, has helped to provide different treatment regimens and two types of experimental speleotherapeutic in accordance with the structure and arrangement of underground spaces in salt, values and environmental experimental pathology (asthma).

- Achievement of biomedical experimental study on laboratory animals with induced disease (blood tests, leucocytes, phagocytosis test, PMN neutrophils test, lymphocyte subpopulation and activation test of T lymphocyte under the action of phytohaemagglutinin in vitro, IgE concentration, cellular parameters of pulmonary fibroblasts, marker of inflammatory process, parameters of hydroelectrolyte metabolism and oxidation-reduction process).

The investigation of salt mine underground occurred in many location in Turda Salt Mine- salt mines or gallery, which have been designated by the unanimous decision of the contracting parties on three levels or one level from „salt soil”.

So, for planned investigations have been designated these locations: Ghizela Mine- for planned investigations, Ghizela Mine – New Crivac, Ghizela Mine – Lower Platform, Rudolf Mine – Monitoring point (near the gondola) Rudolf Mine – Elevator monitoring point, Terezia Mine – at level / the border lake, Frant Iosif Gallery — Inclined Base, Frant Iosif Gallery – Put Rudolf Room, Frant Iosif Gallery – Access intersection with Iosif / Balcon, Control Location – the surface (in front of „Turda Salt Mine entry”).

I. Where conducted these investigations by specialists from INRMFB:

1. Environmental investigations in Turda Salt Mine locations:
   - by Conf.Dr.fiz., CSIII Liviu Enache and Research As. Iulia Bunescu microclimate investigations (temperature, humidity, air flow speed, atmospheric pressure in the salt mine);
   - by CSPII, Dr.b. Iuri (Gheorghe) Simionca investigations of negative and positive ion concentrations in the underground and salt mine aerosol concentration evaluation;
   - by CSPII, Dr.b. Iuri (Gheorghe) Simionca and Dr.b., biochemist pr. Mihai Hoteteu- investigation on CO\textsubscript{2} and other 7-9 gases concentration from the underground, among them being classified as pollutant;
   - by Dr.b., biochemist pr. Mihai Hoteteu – investigation on underground air salinity;
   - by CSII Dr.fiz. Romeo Călin, Executor: SALMROM Laboratory, DFVM Col. 1050, IFIN-HH, contract partner (Subcontract with IFIN-HH)– radioactivity investigation investigații radiation values, dose β / γ radiation, radionuclid concentration in layer („wall”) salt, radon underground concentration;
   - by CSPII, Dr.b. Iuri (Gheorghe) Simionca-microbiological investigation of air (concentration and identify microorganism) and the microbiological wall surface portion and „salt soil”
2. Medico-biological investigations and experiments:

- by CSPII, Dr.b. Iuri (Gheorghe) Simionca, immunologist pr. and Dr.b., biochemist pr. Mihai Hoteteu- experimentally induced disease model (bronchial asthma obtained by ovalbumin antigen sensitized) on Wistar rats for experimental study of speleotherapy cure effect in Turda Salt Mine;
- by CSPII, Dr.b. Iuri (Gheorghe) Simionca - achievement of regimes and types of experimental speleotherapeutic cure specific to quality and status underground factors (microclimatic, physical, biological and microbiological factors and not only) from Turda Salt Mine and status of experimentally induced disease model (bronchial asthma) on Wistar rats in accordance with the structure and the arrangement of underground salt.

3. Medico-biological experimental studies on laboratory animals (Wistar rats) with and without experimental speleotherapeutic cure (after albumin sensitized process):

- by CSPII, Dr.b. Iuri (Gheorghe) Simionca- clinical and medical condition evaluation of Wistar rats groups; to assess clinical status of animals involved in Turda Salt Mine speleotherapeutic courses attended medical and technical personnel from SC Turda Salt Mine SA Durga Wistar Rats trained to care for the experiment.
- by dr.Munteanu Ana and Dr.b.Simionca Iuri- evaluation of leukocytes concentration and blood components
- by Dr.b., biochemist pr. Mihai Hoteteu- investigation of markers of inflammation (protein concentration, protein fractions) and electrolyte metabolism parameters (urinary concentrations of sodium and potassium, sodium balance, potassium and water and the assessment of adrenal hormonal activity
- by CSIII Dr.b. Constantin Munteanu si CS biol.sp. Diana Munteanu- cell biology investigation on pulmonary fibroblasts (concentration and cell morphology, protein concentration, electrophoresis);
- experimental study on nonspecific resistance factors of animal organism
- by CSPII, Dr.b. Iuri (Gheorghe) Simionca, immunologist pr.: evaluation of the immune status and value the sensitized process with ovalbumin, including the types of allergic reaction status

- property assessment of the phagocytic of Staph. aureus by polymorphonuclear neutrophils (PMN) (phagocytosis test) and oxygen-dependent bactericidal function of PMN gran ulocytes by NBT test (Nitrozo-Blau-Tetrazoliu Test);
- immune status evaluation – by investigation of lymphocyte subpopulations- property functional evaluation of proliferation T lymphocytes / blast transformation under phytohaemagglutinin mytogen by lymphocyte transformation in vitro test (TTLB);
- serum cytokines determination – inflammatory mediators and activators macrophages and T lymphocytes (IL-1 α and IL-6, IL-10 and cytokine TNF- α) in bronchial asthma pathology by ELISA immunoassay test (absorption);
- determination of IgE concentration- by Rat-IgE-ELISA-Test and reading results with Multimode Modulus Microplate (absorption, fluorescence and luminescence) TurnerBioSystems (USA ), the values are expressed in ng/ml;
- evaluation of organism sensitized and development of toxico- allergic reactions – by functional property investigation of T lymphocyte proliferation / blast transformation under phytohaemagglutinin mytogen by lymphocyte tranformation in vitro test (TTLB);
II. SOME FINAL RESULTS AND CONCLUSIONS (MEDICO- BIOLOGICAL STUDY OF TURDA SALT MINE UNDERGROUND ABOUT LABORATORY ANIMALS WITH INDUCED PATHOLOGY (BRONCHIAL ASTHMA):

1. Environmental factors promoting natural underground Turda Salt Mine therapeutic potential for use in health and tourism purposes requires a series of activities to achieve pre-scientific study on the quality of these factors and the effect on animal body in the experiment and/or on cells with and without pathology including:
   a) The position of the object in the territory possessing natural factors with potential therapeutic properties and opportunities access.
   b) Accommodation possibilities including a space that could be used for "the treatment basis".
   c) The tourist value of this area, carrying out the tourism circuit and leisure and tourist guides accompanying specific information and scientific knowledge of both tourism and health.
   d) The tourist value of Turda Salt Mine be presented by various classical and modern methods available, this circuit tourism and leisure in salt mine and guides knowledge about both real and specific information of interest and health, and science.
   e) The visitors’ information by guides must contain data on hours and relaxation program, on microclimatic, physico-chemical and microbiological parameters through the tourists understand that descent in salt mine underground galleries worthwhile tourist interest and financial effort and understand that their healthy will not be affected during the presence in mine and the guides must know the emergency medical aid.

The physico-geographical position of Turda Salt Mine with salt mine lakes nearby is good and the access is defined and secured. After the finalisation of planned investigations it was found a solution for accommodation and defined the space for “treatment basis” at the surface.

Was defined the touristic and recreation circuit at surface and in salt mine; the guides know all specific information.

The climate of Tuda area (where the mine is located) is transitional temperate continental without excessive variations (moderate average amplitudes of the main climatic parameters) that print environmentally-friendly features useful both for making tourism and activities such as therapeutic. That contribute to registration of this area in the bioclimate sedative- matter (for sparing) where the human body does not make a special effort of acclimatization and bioclimate not represent special therapeutic contraindications, regardless of the season with no restriction access and parking in the area for those who would follow in Turda Salt Mine for speleotherapy or for tourists- visitors.

2. Microclimatic data and results of the underground studies in Turda Salt Mine made in 80-90 allow the hypothesis “About possible existence of natural factors with therapeutic properties in Turda salt mine”, but this require confirmation from multidisciplinary underground studies, medicobiological experiments on patient with different pathology because it is recognized that not all mines have therapeutic factors and some of them lose their healing properties due to neglect of the underground and anthropogenic pollution.

3. The underground specificity of Turda Salt Mine found in the results of studies is of particular interest and indicate the therapeutic potential of their resorts and tourism, especially the presence of underground natural therapeutic factors, which could be used to treat chronic inflammatory and allergic diseases, respiratory and skin, after all complex studies planned in the contract and after further studies on some groups of patients with asthma and/or other pathologies, the development of methodological recommendations for the use of salt and some specific medical advice and guidance contraindications.

4. During the measurement have found a stable atmosphere with a great stability and homogeneity of microclimatic features (both very small variations from one day to another and between points of measure) which gives the qualities necessary for the interior environmental of several activities types depending on the physical and microclimatic features of this type of environment.

5. The all salt and air samples analysed/measured have no threatening in terms of microbiological, chemical and radioactive.

6. The all values obtained proves that is a clean environment with a limited contact with polluted air from the surface;

7. The anthropogenic activities can change the air purity caused a chemical and microbiological pollution. The results indicate the need for monitoring of underground components in a critical locations in order to prevent pollution and loss of curative properties.

8. Based on: results of the underground study in different mines/gallery from Turda Salt Mine, presence and quality of some components, including potential therapeutic factors three schemes was developed for experimental speleotherapeutic cure on mature and immature Wistar rats with bronchial asthma ovalbumin induced. The pattern of procedures and experimental speleotherapeutic cure (speleotherapy regimes applied) presents a summary based on literature, study results conducted till now
and the results of underground salt mine study from different mines and gallery of Turda Salt Mine.

9. The results obtained form WR investigated groups indicate a series of pathological changes in the body of laboratory animals with experimentally ovalbumin induced bronchial asthma and the positive effect of speleotherapeutic cure on pathophysiological processes and mechanisms of bronchial induced asthma in Turda Salt Mine by applying speleotherapy differentiated and developed regimes.

10. As a result of medical and biological scientific research of underground salt mine, on groups of laboratory animals (Wistar rats) induced by the disease - asthma, conducted in Turda Salt Mine and from the analysis of data and the results of previous scientific research in the field, are found in Turda Salt Mine speleotherapeutic potential natural therapeutic factors, for use in health resorts and tourism, which by the models - differential schemes speleotherapy medico-biological developed and the results of experimental studies found a positive speleotherapeutic effect.

11. The study results are based on experimental data for planning another medico-biological research, this time on patients with bronchial asthma / chronic obstructive and other chronic respiratory diseases, regarding the effect of Turda Salt Mine speleotherapeutic on human subjects, achieving specific treatment methodology Turda Salt Mine, technical and methodological recommendations of various health and specific medical indications and contraindications and therapeutic factors as quality of pathology present status, to advance the necessary documentation to obtain the right to use medical Turda Salt Mine and tourism resorts according to current legislation.
THE ARTIFICIAL AIR IONIZATION EFFECT (NEGATIVE AND POSITIVE) IN EXPERIMENT ON SOME HEMATOLOGICAL PARAMETERS OF WISTAR RATS *

Iu. Simionca, L.Enache
National Institute of Rehabilitation, Physical Medicine and Balneoclimatology

1. INTRODUCTION

1.1. General considerations on the air ionization

The air near the ground, where the most organisms live, is characterized by physical-chemical and biological properties. All these factors (e.g. temperature, humidity, air ionization, etc) perform certain roles and any quantitative and qualitative change, beyond certain limits, are felt on the body in one form or another.

From the physical factors of the air, the electrical power includes, in turn, electrical conductivity, electric field, electrical potential gradient, thunderstorms, air ionization, atmospheric which manifests itself differently in beautiful weather (low cloud, little wind, no precipitation) or the disturbed weather (storm).

The most common electricity of beautiful weather, is characterized by a multitude of meanings with has direct or indirect effects on the living world, favorable or unfavorable, perceptible or not, depending on the intensity, duration or frequency of manifestation of that power factor.

A special place of these biometeorological factors is occupied by the category natural air ionization.

The first observations on the existence of gaseous ions in air have been made since the early twentieth century 30 (German physicist Panthenier Ladenburg and French), thorough research and then resumed after the 50s of various collective (including French physicist J. Bricard, University of Paris). They have highlighted the link between low ion content in the air and micropopulation atmosphere and that lack almost daily a minimum amount of negative ions of oxygen from small places of daily activities constitute a cause of an inevitable occurrence of disorders, often severe, health status.

Thus, it is confirmed that the presence of ions in atmospheric air is essential for life, since their content was found to decrease below a certain threshold value (or worse, their absence) has a negative impact on living organisms (or even their death). A large number of subsequent research have confirmed the link between this parameter of air power and a range of biological effects manifested at various stages of organization in the world live at the cellular level to the body. These influences are possible because living organisms manifests itself in a very large number of phenomena such as electricity with essential biological role (e.g., biochemical reactions, transmembrane transport of the substance of the nervous impulse propagation and others).

However, based on these findings, it was concluded that under the atmosphere of ionized artificially controlled exposure, can ensure relatively easy conditions for recovery of these environmental factors influence by induction of favorable therapeutic treatment on the body.

1.2. Theoretical aspects of natural and artificial air ionization.

The air ionization is the result of a number of physical factors on the one side, and air molecules, on the other side.

The main physical factors of ionization ambient air generators are represented by the natural radioactive elements from soil and air, and cosmic radiation, corpuscular radiation (a, b) and electromagnetic (g, X-ray) emitted directly or indirectly provides energy for ionization of molecules neutral gas and water vapor in the atmosphere.

The molecules ionization phenomenon is complex and takes place in several stages, which can generates various air ions at the end of this. They can be both polarities (positive or negative) and may have different dimensional distributions and electrical mobility, which makes possible the classification of air ions in several categories (low, intermediate and high).

In the initial phase, primary ions are produced, such as those of oxygen in the form:

\[ e^- + O_2 + (M) \rightarrow O^- + (M) \]  
(2.1)

where \( e^- \) is an electron-extracted (by ionizing radiation) from a neutral molecule, and M is any molecule, that takes place the reaction (1).

If electrons have sufficient kinetic energy, then other ions may appear, such as:

\[ e^- + CO_2 \rightarrow O^- + CO \]  
(2.2)

or:

\[ e^- + H_2O \rightarrow H^+ + OH^- \]  
(2.3)
and if \( O^+ \) still has enough energy, can produce a reaction with this form:

\[
O^+ + H_2O \rightarrow OH + OH
\]  

(2.4)

In a later stage the primary ions, associated with other molecules or undergo reactions that lead to other types of ions, such as:

\[
OH + H_2O + X \rightarrow OH(H_2O) + X
\]  

(2.5)

where \( X \) is a certain molecule.

Following this suite of reactions, in the atmosphere may occur gaseous ions as: \( H^-(H_2O), (H_2O)^+ \), \( (H_2O)\_n \), \( OH(H_2O)\_n \) and others.

The ions categories and their concentrations existing in free atmosphere depend on: the intensity (energy) of the ionizing agent, local weather conditions, the degree of pollution of of atmosphere, the secondary physical ionised agents, geological and geographical location of measurement. Normally, air ions concentrations are between 500 - 1000 ion/cm\(^3\) in unpolluted areas outside cities, while in the city fall below 500 ion/cm\(^3\).

Among these ionized secondary agents fragmentation processes of water have particular importance (breaking into small particles, spraying by fine jets of water, air bubbles breaking film from water surface evaporation process, breaking the ice crystals by the collision and others) - known processes and physical phenomena in the physics of the atmosphere, and the passage of atmospheric fronts. In these way can be generated positive and negative electric charges (depending on the chemical composition of water or material, as well as other factors), taken then the microparticles that arise these processes and phenomena.

They manifest, for example, at shore sea (under the sea foamy waves or shore), near a waterfall (50,000 ions/cm\(^3\)), a fountain or a stormy mountain brook (8000 ions / cm\(^3\)) in the rain, the blizzards and more.

Between the action of general factors and that how lead to ions recombination (destruction) is established a certain balance, reflected in a certain air ionization regime in time and place considered.

In addition to natural ionization, through various types of generating equipment can be obtained (in limited areas) ion concentrations of both polarities that can reach very large values, to several million ions / cm\(^3\).

There are a wide variety of air ion generators, with intensities and for different destinations, particularly to ensure the higienic character of the air. Principle of their construction and operation is very diverse, from the use of radioactive elements, devices and solutions for spraying particles or substances to that of electro rivers.

These latter system is based on producing a sufficiently intense electron flow which is immediately captured (less than one millionth of a second), mainly by oxygen molecules, making them negative ions of oxygen. Further, by trapping a few molecules of water, they become small negative ions, with identical properties to those found in nature (provided that they do not generate ozone and nitrogen oxides - considered cytotoxic peroxide).

These generators of air electricity ensures not only environmental pollution (by trapping, precipitation and sedimentation of a large number of particles of different sizes and natures, living or inert, solid or liquid), but also a bactericidal (germicidal) whereas, electrokinetic blocking mechanisms essential to the cell membrane, causing rapid death negative air ions or inactivate all existing pathogens in the air under artificial ionization (actually observed in many bacteriological research, by scientific collective Finnish, North American and Russian but also from other countries).

### 1.3. Biological effects and therapeutic importance of air ionization

As we seen above, the air subject to artificial ionization, which provides a sufficient density of negative ions is more salubrious than one natural.

This electrical parameter of air also presents other facets with significant biological importance. The influence of the ions start mainly from the pulmonay alveolus level and in a lesser extent from the skin.

The excess of negative electric charges carried by ions interact with both sensory nerve endings in the alveoli, and a series of blood components (RBC, some colloids), directly altering their electrical properties and stability and indirectly affecting other properties of various organic structures through numerous biochemical reactions that contribute to increased metabolism.

The studies and the research conducted till now indicates many biological and therapeutic effects. Among those most important influences are mentioned: physical and chemical properties of...
blood (low VSH's increase colloidal stability of the blood serum protein, albumin-globulin ratio decreased due to increase in the quantity globulin, blood pH change to alkaline, reducing the amount of sugar, decrease the accumulation of lactic acid, lowering 5 - hydroxytryptamine and others), cardio-vascular (blood pressure and cardiac pulsations number), neuro-motor system (motor nerves decrease the impact on functional status skeletal muscle, influences on EEG, disappearance of headaches, dizziness, insomnia, etc.), respiratory system (enhanced gas exchange, improving asthma bonsai, slow breathing rate and breathing pauses lengthened), skin (pain killers produced by burning, promoting healing wounds, improving the body's defense response and increase resistance to acute and chronic infectious diseases), endocrine system (regulatory effect, stimulating the formation of vitamins and their accumulation in the blood).

They also observed a number of general effects (increased appetite and, consequently, increased body mass, increasing thoracic volume, improving human performance, individuals become calmer) and bactericidal (bacteria decreased toxicity and developmental delay, accelerate death microbes, reduction of injuries microflora).

Therapeutic contraindications are few and relate to situations rarely encountered in practice. Most often the positive ions, have actions and opposite effects than small negative ions (negative and unpleasant effects experienced by subjects due to, for example, release of serotonin).

Despite the fact that some issues are still under study, the favorable results obtained in recent years have enabled the gradual introduction and successful recovery of negative therapy of air ionization of a large number of diseases, especially abroad, but unfortunately in an insignificant position in our country.

2. MATERIALS AND METHODS FOR STUDY

To achieve the objectives of the study were selected 47 white laboratory Wistar rats line (Wistar Rats - WR), male, divided into three series:

- Series I included 3 homogeneous groups, as follows: Lot 2 - composed of 7 such animals without injury infection and subjected to negative air ionization with concentration of about 15,000 / cm³ particles / cm³ concentration (7 animals), lot 7 - animals with 16.3 cm² injuries infected with microorganisms mentioned, and subject to negative air ionization of about 230,000 / cm³ concentration (9 animals), lot 9 animals with injuries of 16.3 cm², infected and unionized (7animal), lot 10 - animals with injuries of 20.1 cm², infected without air ionization, investigations after 10 days of infection and the development of inflammatory skin infectious process (7 animals);

Series III includes two homogeneous lots: lot 4 - animals with injuries of 13.6 cm², clean, uninfected, subject to positive air ionization of about 230,000 / cm³ particles concentration (7 animals), lot 8 - animals with injuries of 13.6 cm² infected, subject to the same polarity and concentration air ionization (9 animals).

The animlas were anesthetized with phenoobarbital of 12 mg/ml solution in heparin and then on them back were caused a scaping injury that was infected with Staphylococcus aureus from a pure culture, etiology concentration of 10⁹ / ml, such as the infectious process induced skin inflammation (PII).

Since the 2nd day after the beginnings of inflammatory process series I and day 4 respectively of the animals in series II, were subjected for 4 daily to air ions for 3 weeks; animals were kept under standard conditions biobase.

In the table below are presented: WT groups subjected to study, polarity and artificial air ions concentration generated for application on laboratory animals in the experiment, average area of cutaneous injury and observations regarting to their inflammation with microorganism conditionally pathogenic- Staphylococcus aureus.
Air ionization of cutaneous surface with and without PII was performed using an ion generator with possibilities to generate 15,000-230,000 negative / positive ions / cm³.

Was made the following tests on laboratory animals (WR):

1. The assessment of WBC blood concentration by photon microscopy method Bürker room and expressing of cell number in l/ml (nx10⁻⁹/l sau nx10⁻⁹/ml) and various WBC by photon microscophy of blood blades fixed by May Grünvald methodology and expression of the results in relative (%) and absolute (nx10⁻⁹/l) values.

2. Hematocrit determination by micromethod (Micro Haematocrit Tubes Modulohm A/S, Denmark).

3. RESULTS

Results of hematological investigations on white Wistar rats, with and without inflamed injuries, subject to the action of various schemes with artificial ionization positive and negative polarity were compared with some from literature (Jaskowski, J. and Mysliwski, A., 1986; Guidelines for Collection of Blood from Experimental Animals. University of Minnesota, USA. HTML Document, 2006; Hematological Values for Long Evans Rats. Hematological Values for Wistar-Kyoto Rats. TACONIC. ANTECH diagnosis, 10 Executive Blvd. Farmingdele. Nz 11735. HTML Document, 2006; Puggina Rogato Gustavo, Elite Luciano, 2006).

Analysis of these data allowed to highlight some changes in the immune system. Should be noted that the blood cells WBC have an important role both in inflammation process and the immune system of animals and humans.

According to the results presented in figures 1 of 12 laboratory animals with or without skin and infected injuries, subject to artificial air ionization negative or positive at of 15,000 and 300,000 particles / cm³ concentration was observed some changes in concentration and distribution of various forms WBC blood.

Figure 1 presents the concentration of WBC blood of laboratory animals in the experiment and figures 2-12 the relative (%) and absolute (nx10⁹/l) values of different WBC and those leukocytic indices.

In control and uninfected lots of WR (S2L1-without ionization and injury, uninfected), but subject to negative air ionization with 15,000 particles / cm³ (S1L2) there were no pathological changes in blood WBC concentrations.

It was found a decrease in the concentration of WBC blood of laboratory animals without skin injury and uninfected but subjected to negative air ionization with 230,000 particles / cm³ (S2L3 group) and those with experimental injury skin surface, subsequently infected with suspension of Staph. aureus (lot S2L7).
Figure 1.

Concentrația de neutrofile (nx10^9/l) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

![Graph showing neutrophil concentrations in blood of albino Wistar rats from different lots under artificial ionization.](image)

<table>
<thead>
<tr>
<th>Lot</th>
<th>Value (mean)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1.L1</td>
<td>2.73</td>
<td>0.177</td>
</tr>
<tr>
<td>S1.L2</td>
<td>3.22</td>
<td>0.456</td>
</tr>
<tr>
<td>S2.L3</td>
<td>1.73</td>
<td>0.341</td>
</tr>
<tr>
<td>S1.L5</td>
<td>3.34</td>
<td>0.336</td>
</tr>
<tr>
<td>S2.L9</td>
<td>3.42</td>
<td>0.465</td>
</tr>
<tr>
<td>S2.L10</td>
<td>2.60</td>
<td>0.311</td>
</tr>
<tr>
<td>S1.L6</td>
<td>2.67</td>
<td>0.475</td>
</tr>
<tr>
<td>S2.L7</td>
<td>1.81</td>
<td>0.393</td>
</tr>
<tr>
<td>S3.L4</td>
<td>1.94</td>
<td>0.206</td>
</tr>
<tr>
<td>S3.L8</td>
<td>2.65</td>
<td>0.360</td>
</tr>
</tbody>
</table>

Figure 2.

Concentrațiile leucocitelor sangvine la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionzării artificiale, nx10^9/l

![Graph showing white blood cell counts in the blood of albino Wistar rats from different lots under artificial ionization.](image)

<table>
<thead>
<tr>
<th>Lot</th>
<th>Value (mean)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1.L2</td>
<td>9.8</td>
<td>0.79</td>
</tr>
<tr>
<td>S1.L3</td>
<td>10.1</td>
<td>0.73</td>
</tr>
<tr>
<td>S2.L4</td>
<td>6.7</td>
<td>0.94</td>
</tr>
<tr>
<td>S1.L5</td>
<td>10.9</td>
<td>0.44</td>
</tr>
<tr>
<td>S2.L9</td>
<td>12.3</td>
<td>1.36</td>
</tr>
<tr>
<td>S2.L10</td>
<td>9.8</td>
<td>0.55</td>
</tr>
<tr>
<td>S1.L6</td>
<td>8.7</td>
<td>0.82</td>
</tr>
<tr>
<td>S2.L7</td>
<td>7.4</td>
<td>1.04</td>
</tr>
<tr>
<td>S3.L4</td>
<td>6.3</td>
<td>0.92</td>
</tr>
<tr>
<td>S3.L8</td>
<td>8.3</td>
<td>1.09</td>
</tr>
</tbody>
</table>

Figure 3.

Concentrația (% ) de neutrofile nesegmentate în sânge la șobolanii albi Wistar sub acțiunea aeroionizării artificiale

![Graph showing percentage of unsegmented neutrophils in blood of albino Wistar rats under artificial ionization.](image)

<table>
<thead>
<tr>
<th>Lot</th>
<th>Value (mean)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>5</td>
<td>0.8</td>
</tr>
<tr>
<td>S1.L2</td>
<td>4</td>
<td>1.4</td>
</tr>
<tr>
<td>S2.L3</td>
<td>4</td>
<td>0.5</td>
</tr>
<tr>
<td>S1.L5</td>
<td>7</td>
<td>0.8</td>
</tr>
<tr>
<td>S2.L9</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td>S2.L10</td>
<td>7</td>
<td>0.9</td>
</tr>
<tr>
<td>S1.L6</td>
<td>6</td>
<td>0.7</td>
</tr>
<tr>
<td>S2.L7</td>
<td>6</td>
<td>0.8</td>
</tr>
<tr>
<td>S3.L4</td>
<td>4</td>
<td>0.9</td>
</tr>
<tr>
<td>S3.L8</td>
<td>6</td>
<td>1.3</td>
</tr>
</tbody>
</table>
Similar results were observed to groups of animals subjected to positive artificial air ionization of 230,000 particles / cm³ (lot S3L4), although should be noted that in the presence of infected injuries concentrations of WBC blood (lots S1L6 and S3L8) are just a decrease trend.

The increase of WBC concentration occurred of rats lots with 3.0 cm² 16.3 cm² injuries experimentally infected with microorganisms but unionized (lots S1L5 and S2L9); insignificant of animals after 10 days of infection (S2L10 lot).

The figures 2 to 6 present the neutrophils concentrations (relative - % and nx10⁹/ L) of investigated lots of animals. Compared with control lots (S2L1- without ionization and injuries), the experimental lot S1L2 without infection and subjected to the negative air ionization (15,000 / cm³) of WR in most experimental lots, including those of positive air ionization, were not found pathological changes in relative concentration (%) by blood neutrophils, although should also mention that in animals subjected to negative air ionization with 230,000 particles / cm³, with infected skin injury surface after the experiments with suspension of *Staph. aureus* (lot S2L7) showed a trend of decreasing in neutrophils concentrations (ie 32 + / -4.3 and 24 + / -2.5%).

Significantly reduced values were found in expression of the concentration of blood neutrophils in absolute formative elements (NX10 * 9 / L), especially of animals without skin injury (lot S2L3) and those with skin injury surface after the infection with *Staph. aureus in suspension* (lot S2L7), subjected to negative air ionization 230,000 particles / cm³, and positive artificial air ionization 230,000 particles / cm³ (lot S3L4).

Instead, the trend of increasing absolute concentration of neutrophils (NX10 * 9 / L) in blood was observed on WR with injury batches of 3.0 cm² 16.3 cm² experimentally infected with microorganisms but ionized (lots S1L5 and S2L9), which may be due to nonspecific reaction of the body to infectious-inflammatory process.

Compared with control lots (S2L1- without ionization and injury), the experimental lot S1L2 with uninfected injury and subjected to negative air ionization (15,000 / cm³) on most experimental groups of WR, including those subjected to positive air ionization, were not found pathological changes in relative concentration (%) of blood neutrophils, although we should note that of animals subjected to negative air ionization 230,000 particles / cm³, with infected skin injury surface after the experiments with suspension of *Staph. aureus* (lot S2L7) showed a trend of decreasing in neutrophils concentrations (ie 32 + / -4.3 and 24 + / -2.5%).

Significantly reduced values were found in expression of the concentration of blood neutrophils in absolute formative elements (NX10 * 9 / L), especially of animals without skin injury (lot S2L3) and those with skin injury surface after the infection with *Staph. aureus in suspension* (lot S2L7), subjected to negative air ionization 230,000 particles / cm³, and positive artificial air ionization 230,000 particles / cm³ (lot S3L4).

Instead, the trend of increasing absolute concentration ( neutrophils NX10 * 9 / L) in blood was observed on WR with injury batches of 3.0 cm² 16.3 cm² experimentally infected with, which may be due to nonspecific reaction of the body to infectious-inflammatory process.

Significant changes were observed in the fractional concentrations of nonsegmented and segmented neutrophils (polymorphonuclear neutrophils - PMN). (Figures 3 and 4).
Figure 4.

Concentrația de neutrofile nesegmentate (n\textsuperscript{9}*1/l) în sânge la șobolanii albi Wistar sub acțiunea aeroionizării artificiale

![Graph with data points and error bars]

Valori medii

Deviație standard


0.49  0.43  0.30  0.79  0.72  0.87  0.48  0.43  0.26  0.50

0.096 0.151 0.065 0.102 0.154 0.094 0.080 0.071 0.063 0.095

Figure 5.

Concentrația (% de neutrofile segmentate în sânge la șobolanii albi Wistar sub acțiunea aeroionizării artificiale

![Graph with data points and error bars]

Valori medii

Deviație standard


23  27  21  23  22  22  25  27  27  26

3.7  5.4  3.3  2.6  3.2  2.5  2.8  2.9  2.6  2.8

Figure 6.

Concentrația de neutrofile segmentate (nx10\textsuperscript{9}*1/l) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

![Graph with data points and error bars]

Valori medii

Deviație standard


2.24  2.77  1.43  2.55  2.70  2.13  2.19  1.38  1.68  2.74

0.203 0.551 0.301 0.320 0.491 0.292 0.419 0.363 0.195 0.395
We should note that WR from S1L5 lots (without ionization and about 3.0 cm² infected injuries with Staph. aureus), S2L9 (no ionization, but infected injuries of 16.3 cm² average) and S2L10 (without ionization, after 10 days with infected injuries of 20.1 cm² average), so in the presence of acute inflammatory and infectious process, lasting 10 days, the concentration of the nonsegmented neutrophils (P < 0.05) expressed both in relative (%) and absolute values (NX10 * 9 / L) was significantly increased, observing the forms and cases of immature and neutrophil precursors. Under the action of negative air ions of about 15,000 particles / cm³ in the blood of investigated animals from different experimental lots were not found pathological changes in relative values (%). Of animals from S1L6 lots (with infected injuries, subject to negative air ionization of 15,000 / cm³ concentration) was observed only the trend of increasing in concentration of the nonsegmented neutrophils (P > 0.1), expressed in relative values (%). Were found low absolute values (NX10 * 9 / L) of nonsegmented blood neutrophil to WR from lots S2L3 (uninfected, subject to negative air ionization of 230,000 particles / cm³ concentration) and S3L4 (uninfected, subject to positive air ionization of 230,000 particles / cm³ concentration) and for S2L7 and S3L8 lots (with infected injuries, subject to positive and negative air ionization of 230,000 / cm³ concentration) - the trend of relative concentration increasing (%) of nonsegmented neutrophils (P > 0.1).

In terms of concentration variations of segmented neutrophils (PMN), it is noteworthy that at WR of 3.0 cm² infected with Staph. aureus but uninfused, and on animals with infected injuries of 16.3 cm², but without applying air ionization (lot S2L9) was found an increase of PMN neutrophile in blood concentration.

After the negative air ionization of about 15,000 / cm³ particles (lot S1L2) at WR without injuries and uninfected has been found a growing trend in the number of PMN cells in the blood. After the negative air ionization of about 230,000 / cm³ particles there was a relative and absolute decrease in the number of PMN neutrophils in blood from both the WR group with 16 cm² injuries infected with Staph. aureus (group S2L7) as well as from animals uninfected and without injuries (lots S2L3 and S2L7).

The trend of the number of these cells blood decreasing was observed at uninfected WER without injuries, but subjected to positive air ionization of about 230,000 / cm³ (lot S3L4).

The figures 7 and 8 present the relative (%) and absolute (nx10⁹/L) concentration of blood eosinophils on WR in the experiment.

On WR with injuries and infection induced (S3L8 lot) was found a increasing trend of relative eosinophils (%) in the blood.

In the blood of the animals from infected and uninfected, with and without injuries lots subjected to negative air ionization of about 15,000 / cm³ (lot S1L6) and 230.000 / cm³ (lots S2L3 și S2L7) concentration and from uninfected and without injuries lot subjected to the same concentration of positive air ionization (lot S3L4) was found downward trend in relative number (%) of eosinophils, absolute number (NX10 * 9 / L) of these cells was significantly lower.

The concentration of relative monocytes (%) from the blood doesn't show a significant variation. The analysis of absolute concentration results (NX10 * 9 / L) of the blood monocytes investigated to WR is shown in figure 9.

Some changes of monocytes concentration, namely a significant decrease in absolute number (NX10 * 9 / L) of blood monocytes were observed at WR from uninfected or infected injuries lots (lots S2L7, S3L4) subjected to negative and positive air ionization.

Similarly, was mentioned a downward trend in the number of these blood cells at uninflamed animals subjected to negative air ionization (lot S2L3). For the immune system of human and animals an important role is played by lymphocyte cells. It was found that the relative concentration (%) of WR blood lymphocytes from different investigated groups didn't suffer significant changes. Decreasing trend of blood lymphocyte was observed only for infected animals subjected to positive maximum air ionization of about 230,000 / cm³ (lot S3L8) (respectively 56 +/- 2.1 % and 66 +/-1.7 - 61 +/-3.0 %).

As regarts to the absolute values of results (NX10 * 9 / L) was found (figure 10) that at WR with skin injuries and inflammatory process (S2L9 lot of animals with 16.3 cm² injuries infected with Staph. aureus) observed trend of increasing the number of lymphocytes in the blood and at those with or without infected injuries and subject to positive air ionization of about 230,000 particles / cm³ (lots S3L4 and S3L8), has been a considerable decrease in the concentration of cell lymphocyte.

Similarly, was mentioned a downward trend in the absolute number of blood lymphocytes was mentioned to uninfected WR subjected to subject to maximum negative air ionization of 230,000 particles / cm³ (lot S2L3).

We also should calculate the mathematical relationships on status and inter-relations between different leukocytes.
Figure 11 presents the data analysis of the mathematical ratio "nonsegmented neutrophils / segmented neutrophils and figure 12 the report" neutrophil / lymphocyte".

Figure 7.

Concentrația (%) de eozinofile în sânge la șobolanii albi Wistar în diferite loturi sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori medii</th>
<th>Deviație standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>S1.L2</td>
<td>1</td>
<td>1.0</td>
</tr>
<tr>
<td>S2.L3</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>S1.L4</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>S2.L5</td>
<td>0</td>
<td>0.8</td>
</tr>
<tr>
<td>S2.L6</td>
<td>5</td>
<td>1.0</td>
</tr>
<tr>
<td>S2.L7</td>
<td>4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Figure 8.

Concentrația de eozinofile (nx10^9/l) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori medii</th>
<th>Deviație standard</th>
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<tbody>
<tr>
<td>S2.L1</td>
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<td>0.098</td>
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<tr>
<td>S1.L2</td>
<td>0.57</td>
<td>0.122</td>
</tr>
<tr>
<td>S2.L3</td>
<td>0.32</td>
<td>0.060</td>
</tr>
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<td>0.61</td>
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</tr>
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<td>0.69</td>
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<td>S2.L6</td>
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</tr>
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<td>S2.L7</td>
<td>0.44</td>
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<tr>
<td>S3.L1</td>
<td>0.34</td>
<td>0.066</td>
</tr>
<tr>
<td>S3.L2</td>
<td>0.27</td>
<td>0.086</td>
</tr>
</tbody>
</table>

Figure 9.

Concentrația de monocite (nx10^9/l) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori medii</th>
<th>Deviație standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>0.51</td>
<td>0.137</td>
</tr>
<tr>
<td>S1.L2</td>
<td>0.55</td>
<td>0.155</td>
</tr>
<tr>
<td>S2.L3</td>
<td>0.36</td>
<td>0.068</td>
</tr>
<tr>
<td>S1.L4</td>
<td>0.60</td>
<td>0.122</td>
</tr>
<tr>
<td>S2.L5</td>
<td>0.53</td>
<td>0.182</td>
</tr>
<tr>
<td>S2.L6</td>
<td>0.41</td>
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</tr>
<tr>
<td>S2.L7</td>
<td>0.40</td>
<td>0.077</td>
</tr>
<tr>
<td>S3.L1</td>
<td>0.35</td>
<td>0.071</td>
</tr>
<tr>
<td>S3.L2</td>
<td>0.30</td>
<td>0.099</td>
</tr>
<tr>
<td>S3.L3</td>
<td>0.53</td>
<td>0.081</td>
</tr>
</tbody>
</table>
Concentrația de limfocite (x10^9/l) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori media</th>
<th>Deviație standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>6.05</td>
<td>0.738</td>
</tr>
<tr>
<td>S1.L2</td>
<td>5.78</td>
<td>0.572</td>
</tr>
<tr>
<td>S2.L3</td>
<td>4.31</td>
<td>0.639</td>
</tr>
<tr>
<td>S1.L5</td>
<td>6.39</td>
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</tr>
<tr>
<td>S2.L9</td>
<td>7.67</td>
<td>0.969</td>
</tr>
<tr>
<td>S2.L10</td>
<td>6.05</td>
<td>0.400</td>
</tr>
<tr>
<td>S1.L6</td>
<td>5.15</td>
<td>0.347</td>
</tr>
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<td>S2.L7</td>
<td>4.93</td>
<td>0.641</td>
</tr>
<tr>
<td>S3.L4</td>
<td>3.79</td>
<td>0.609</td>
</tr>
<tr>
<td>S3.L8</td>
<td>4.64</td>
<td>0.569</td>
</tr>
</tbody>
</table>

Valoarea raportului neutrofile nesegmentate (%) / neutrofile segmentate (%) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori media</th>
<th>Deviație standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>0.22</td>
<td>0.05</td>
</tr>
<tr>
<td>S1.L2</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>S2.L3</td>
<td>0.21</td>
<td>0.06</td>
</tr>
<tr>
<td>S1.L5</td>
<td>0.31</td>
<td>0.06</td>
</tr>
<tr>
<td>S2.L9</td>
<td>0.27</td>
<td>0.10</td>
</tr>
<tr>
<td>S2.L10</td>
<td>0.32</td>
<td>0.06</td>
</tr>
<tr>
<td>S1.L6</td>
<td>0.22</td>
<td>0.03</td>
</tr>
<tr>
<td>S2.L7</td>
<td>0.31</td>
<td>0.08</td>
</tr>
<tr>
<td>S3.L4</td>
<td>0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>S3.L8</td>
<td>0.24</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Valoarea raportului neutrofile (%) / limfocite (%) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori media</th>
<th>Deviație standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>0.46</td>
<td>0.08</td>
</tr>
<tr>
<td>S1.L2</td>
<td>0.56</td>
<td>0.11</td>
</tr>
<tr>
<td>S2.L3</td>
<td>0.40</td>
<td>0.07</td>
</tr>
<tr>
<td>S1.L5</td>
<td>0.52</td>
<td>0.06</td>
</tr>
<tr>
<td>S2.L9</td>
<td>0.45</td>
<td>0.06</td>
</tr>
<tr>
<td>S2.L10</td>
<td>0.46</td>
<td>0.06</td>
</tr>
<tr>
<td>S1.L6</td>
<td>0.51</td>
<td>0.05</td>
</tr>
<tr>
<td>S2.L7</td>
<td>0.36</td>
<td>0.07</td>
</tr>
<tr>
<td>S3.L4</td>
<td>0.52</td>
<td>0.06</td>
</tr>
<tr>
<td>S3.L8</td>
<td>0.57</td>
<td>0.07</td>
</tr>
</tbody>
</table>
It was found that from WR lots with infected injuries without ionization (lots S1L5, S2L9, S2L10), the lot with infected injuries subjected to negative air ionization with increased concentration of about 230,000 particles / cm³ (lot S2L7) the value ratio "nonsegmented neutrophils / neutrophil segmented" from blood has been increasing and tends to increase which indicates a neutrophil response to inflammatory process induced, characterized by the development of young or immature forms, and in group of animals with infected wounds but subject to positive air ionization of about 230,000 particles / cm³ (S3L4 lot) - the tendency of decreasing the value of that report, which could be a result of suppression of young or immature nonsegmented neutrophils.

The data from figure 12 indicate the presence of a increasing trend of "neutrophil / lymphocyte" ratio at infected WRunionized (lot S1L5) and those with infected injuries subjected to negative air ionization of 15,000 particles / cm³ (lot S1L2), which may represent a stimulation of PMN neutrophils system. Of animals with infected injuries and subjected to negative air ionization of 230,000 particles / cm³ was observed trend of decreasing the value of that report.

It was also found that the relative values (%) of hematocrit from WR blood didn’t differ from experimental lots. The results obtained from laboratory animals uninfected and without injuries subjected to negative air ionization of 230,000 particles / cm³ (lot S2L3 - 40,43 +/- 1,27 %), and from those subjected to positive air ionization with the same increased concentration (lots S3L4 - 40,00 +/-1,15% si S3L8), shows a tendency to decrease of hematocrit value compared with the control group animals (lot S2L1) and infected lots with or without air ionization of 15,000 particles / cm³ (lots S2L9 and S1L6) (respectively 40,43 +/-1,27 % si 40,00 +/-1,15% - 41,44 +/-1,13%compared with 44,14 +/-1,77 % - 46,71 +/-1,11% - 46,78 +/-1,10%, P <0,05).

4. DISCUSSIONS AND CONCLUSIONS.

The analysis of the results indicate a redistribution of the relative number and total concentration (NX10 * 9 / L) in blood leukocytes and various forms of leukocyte, especially neutrophil PMN, young-nonsegmented neutrophils, and lymphocytes - as a result of an inflammatory infectious process and a organismic non-specific response to infection and the air ionization effect with different polarity and concentration.

The data obtained are both practical and fundamental research interest and indicate the need for development of studies in this area.

*Results obtained in phase IV(Responsible– CPII Dr.b. Simionca Iuri), Project Nr.466/2004 – 2007(Project Manager- Conf. Dr. fiz. Enache Liviu)

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3. Botea Simona, Mocanu Mihaela, Filipescu C., Tarniță Georgeta, Enache Liviu, Simionca Iu,(2005): The effect of the artificial aeroionization on the factors with atheromatose potential.Scientific Works (Lucrări științifice), Faculty of Veterinary Medicine, University of Agronomical sciences and veterinary medicine, Bucharest, 2005, C Series XLVIII, p.539-551


A special place in the category of balneometeorological factors is occupied by the natural air ionization.

The first observations on the existence of gaseous ions in the air have been made since the early twentieth century (German physicist Landerburg and French- Panthenier), and then repeated and through after the 50 of various collective (including French physicist J. Bricard, from University of Paris). They highlighted the link between low ion content in the air and atmosphere micropopulation and the fact that the lack almost daily of a minimum amount of a small negative ions of O₂ from places of daily activities represent a case of occurrence of unavoidable disturbance on health status, often severe.

A large number of subsequent research have confirmed the link between this electrical parameter of air and a large biological effects manifested at various stages in world living organization, from cellular level to the body. These influences are possible because living organisms manifests itself a very large number of phenomena such as electricity, with essential biological role (e.g., biochemical reactions, transmembrane transport of the substance, the nervous impulse propagation and others). However, based on these findings, it was concluded that under the artificially ionized atmosphere by controlled exposure, can provide relatively easy conditions for recovery of these environmental factors by induction a favorable therapeutic treatment on body. The ions influence start mainly from alveoli and in a lesser extent from skin.

The excess of negative electric charges carried by ions interact with both sensory nerveendings in the alveoli, and a series of blood components (RBC, some colloids), changing directly their electrical properties and stability and indirectly affecting other properties of various organic structures through numerous biochemical reactions that contribute to increased metabolism.

The studies and researches conducted till now indicate the existence of numerous biological effects. Although some investigation on the effects of negative and positive air ionization have been done during the years 1950-60 (F. Verzar, 1955; A. P. Krueger, R. F. Smith and Ing Gan Go, 1957; A. P. Krueger, R. F. Smith, 1958, 1960; L. L. Vassiliev, 1960; A. L. Tchijevski, 1963) this problem is current because of the development of different technologies which affect the environment (Ardelean I., Barnea M., 1972, Enache L. § coaut., 1972 – 2005; Simionca Iu.M., Gorbkeno P.P., Gorbkeno V.P., 1994 - 1995);

The studies on the effect of negative and positive air ionization on the nonspecific resistance of the organism and immune status whose decrease may cause various chronic infections with normal, conditionally pathogenic microflora or latent viruses (Pierson, 1993; Taylor et al., 1997) have an special interest.

The action of the negative natural air ions on the human and animal organism is a positive factor (Boulatov, P. C., 1968; Jones D.P. and al., 1976; Simionca Iu.M., Gorbkeno P.P., Gorbkeno V.P., 1980; Botea Simona și al., 2005; Enache Liviu, Hoteteu M., Rogojan Rodica, Simionca Iu. și al., 2005; Căpitanco, Simionca Liviu, Simionca Iu. și al., 2005; Sîrca Ancuța și al., 2004; Tarniță Georgeta și al., 2005).

Experimentally was found that in cases with insufficient negative air ions in the body of laboratory animal the pathogens persist in different cavities of organs, as a result of disruption of the phagocytosis process (Tchijevski A. L., 1963). It is known that natural negative air ionization does’t have high values, with variations of about 200 to 15,000 particles / cm³.

The emergence of opportunities to use artificial air ionization in research and practical allowed developing and launching further studies, including microbiology, infectious-inflammatory process, but also immunology studies, although it should be mentioned that immune investigations were often humoral and from the cellular spectrum –some on the phagocytosis process on the immune status of animals and humans was rare (Kornblueh, I. H.,1973; Laza, V.,1996).

From the point of negative air ionization, effects on microorganisms and inflammatory process the studies of AP. Krueger and collaborators have a particularly interest (1957- 1985), Marin et al., 1989).

Are described some investigation about the beneficial effect of negative air ionization on the inflamed experimentally induced injuries for dogs and cats (A.K.Guman și Z.P. Tsapotsnikova, 1959), as well as chronic injuries of humans (Minehart J.
Some studies were dedicated to the desensitizing effect of body under the action of negative air ions (Boulatov, P. C., 1968). In Antarctica was found a positive effect of negative air ionization on the cellular immune system (Williams, D.L., Clime, A., Muller, H.K. and Lugg, D.J. 1986). The negative air ionization is one of the components of the therapeutic mechanism of caves and salt mines microclimate (Simionca Iu.and al., 1997, 1999, 2005; Enache Liviu, Filipescu C., Simionca Iu. (Ghe.) și al., 2005).

Positive air ions cause depression, insomnia, headaches, rashes, acute asthma attacks, affects the normal activity of the thyroid glands (Gualtierotti, 1968), an opposite effect of negative air ions (Livanova et al., 1999).

2. MATERIALS AND METHODS USED FOR THE STUDY.

To achieve the objectives of study were selected a total of 47 laboratory white line Wistar rats (Wistar Rats - WR), male, divided into three series:
- Serie I included 3 homogeneous lots, as follows: Lot 2 - composed of seven such animals without injury, uninfected and subjected to negative air ionization of about 15,000 particles / cm³ concentration; lot 5 - of 9 animals with injuries of skin surface to which equal to 3.0 cm² infected with Staphylococcus aureus, unionized, Lot 6 -of 9 animals with injuries of 3.75 cm² infected with that microorganisms;
- serie II – 3 homogeneous lots: lot 1 – animals without injury, uninfected and without ionization (7 animals); lot 3 – animals without injury, uninfected and subjected to negative air ionisation of about 230,000 particles / cm³ concentration (7 animals); lot 7- animals without injury of 16,3 cm², infected with that microorganism and subjected to negative air ionization of about 230,000 particles / cm³ concentration (7 animals); lot 9 – animals with injury of 20,1 cm², infected, unionized, investigated after 10 days from infection and the development of inflammatory skin infectious process (7 animals);
- serie III - with 2 homogeneous lots: lot 4 – animals with injury of 13,6 cm², uninfected, subjected to positive air ionization of about 230,000 particles / cm³ (7 animals); lot 8 – animals with injury of 13,6 cm², infected, subjected to the same Table 1. Lots of WR white rats subjected study concentration and polarity of air ionization (9 animals). The animals were anesthetized with pentobarbital at a concentration of 12 mg / ml heparin solution and then on the back of them were caused a injury by scraping who was infected with a pure culture of Staphylococcus aureus of 10⁹ / ml etiologic concentration, thus was induced the infectious skin inflammation process (PII).

From the 2nd day after the start of inflammation process of series I and day 4 respectively for series II, lots were subjected to ions for 4 hours / day for three weeks, animals were kept under standard conditions of biobase.

Table 1: The process of phagocytosis of polymorphonuclear granulocytes (PMN).

a. Phagocytic activity against Staph. aureus / number of phagocytic cells (% and NX10⁹ * 9 / l blood);

b. Number of phagocytized microbial cells (Staph.aureus);

c. Killing Effect / Number of microbial cells in decay phasefrom phagocit cytoplasm (%).

For performing phagocytosis test was used pure PMN granulocyte cells suspension, obtained by sedimentation forced method in ficoll- omnipac gradient with density 1077; pure culture of Staph. aureus was standardzed after concentration. Phagocytosis test was performed according to the EAKost methodology (1975), with methodical recommendations proposed by S. Wood, A. White (1978).

Evaluation of oxygen-dependent bactericidal function of PMN granulocytes (oxidative metabolism of phagocytes) – by NBT test (Blau-nitroso-tetrazolium test) which is based on the Blau-tetrazolium reduction of substance in the cells by the action of insoluble formasan NADF oxidase. NBT test was performed according to method B. Park et al., (1968) and methodical recommendations of E.F. Cernușenko. (1988). The result was read by photon microscopy with immersion and presented by the number of granulocytic cells formasan positive PMN (% and NX10 * 9 / l blood).

3. For immunological tests on populations and subpopulations of lymphocytes, including pure were obtained lymphocyte suspension cells using the centrifuge technique and methodology of heparinized blood in Ficoll gradient (Feinchemie Approach, Austria) and Omnipac (Maging Nicomedia, Norway) d. 1077 by the method C. Bar. (1973) and J. Bach et al. (1968) and methodical recommendations of E.F. Cernușenko et al. (1988) and A. Olinescu Angela Dolganiuc (2001), C. Bar. (2002).

4. The assessment test cells of T lymphocytes from Wistar rats was performed after the methodological of M. Jondal, G. Klein (1973) and J. Bach et al. (1974) by test E-Rosette forming cells - E-RFC (rosette-forming lymphocyte cells with sheep erythrocytes - "E-CLFR" - the classic test for assessing the T-cell) E-test CLFR, 29 C and (concentration of lymphocytes T 29 C ° - T-helpers,% and NX10 * 9 / l blood) - cells forming rosettes "high and low affinity “ (Helper and suppressor), and test E-CLFR, 45 ° C (45 C concentration and T lymphocytes - cytotoxic and NX10% 9 / 1 blood), using the methodology of the test micromethod recommended by Iu.Simionka (1985, 1989) and E.F. Cernușenko. (1988) and A. Olinescu Angela Dolganiuc (2001). Reading the results was performed by photon microscopy after Giemsa stained blade taking into account the methodological recommendations J. Evans et al (1975).

5. The test of lymphocytes blast transformation (TTLB) in phytohaemagglutinin activation. The test was performed according to classical methodologies (by F. Bach, K. Hirschorn, 1963), adapted for working with pure cultures of lymphocytes (after Iu.Simionca, 1985, 1989) and morphological evaluation of activated lymphocytes and transformed into blasts, the blades was read by photon microscope.

### Table 1: Specific characterization of WR lots

<table>
<thead>
<tr>
<th>Name Lot WR</th>
<th>Marking lots</th>
<th>Ionization type</th>
<th>Particules-ions concentration</th>
<th>WR with and without injuries</th>
<th>Specific characterization of WR lots</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2L1</td>
<td></td>
<td>Without ionization</td>
<td>0</td>
<td>Without injury</td>
<td>Control lot</td>
</tr>
<tr>
<td>S1L2</td>
<td></td>
<td>Negative</td>
<td>15,000</td>
<td>Without injury</td>
<td>Uninfected</td>
</tr>
<tr>
<td>S2L3</td>
<td></td>
<td>Negative</td>
<td>230,000</td>
<td>Without injury</td>
<td>Uninfected</td>
</tr>
<tr>
<td>S1L5</td>
<td></td>
<td>Without ionization</td>
<td>0</td>
<td>3.0</td>
<td>Infected with Staph. aureus</td>
</tr>
<tr>
<td>S2L9</td>
<td></td>
<td>Without ionization</td>
<td>0</td>
<td>16.3</td>
<td>Infected with Staph. aureus</td>
</tr>
<tr>
<td>S2L10</td>
<td></td>
<td>Without ionization</td>
<td>0</td>
<td>20.1</td>
<td>Infected with Staph. aureus; investigated after 10 days</td>
</tr>
<tr>
<td>S1L6</td>
<td></td>
<td>Negative</td>
<td>15,000</td>
<td>3.75</td>
<td>Infected with Staph. aureus</td>
</tr>
<tr>
<td>S2L7</td>
<td></td>
<td>Negative</td>
<td>230,000</td>
<td>16.3</td>
<td>Infected with Staph. aureus</td>
</tr>
<tr>
<td>S3L4</td>
<td></td>
<td>Positive</td>
<td>230,000</td>
<td>13.6</td>
<td>uninfected</td>
</tr>
<tr>
<td>S3L8</td>
<td></td>
<td>Positive</td>
<td>230,000</td>
<td>13.6</td>
<td>Infected with Staph. aureus</td>
</tr>
</tbody>
</table>
subject to positive air ionization of 230,000 particles / cm$^3$ (lots S3L4 and S3L8) - a substantial decrease in relative number of phagocytic cells (P < 0.05).

From point of air ionization effect on phagocytic activity is noted that on laboratory animals infected, with injuries (area of 3.0 and 16.3 cm$^2$) but non-ionized (lots S1L5, S2L9) was observed a trend of increasing absolute number of phagocytic PMN; analog – at animals without infection but subjected to negative air ionization 15,000 particles / cm$^3$ (lot S1L2).

Under the negative and positive air ionization of 230,000 particles / cm$^3$ concentration on without injuries and uninjured animals and those with skin injuries infected with Staph. aureus was found significantly reduced values (lots S2L3 and S3L4) or a downward trend of these values (lots S2L7 and S3L8) of the absolute number of PMN phagocytic cells.

Killing Effect values of phagocytized microorganisms (the destruction of microorganisms) by PMN phagocytic cells (figure 15) were found maximum (in the control group data) to the WR from the control lot (S2L1), to those without injury but subject to negative air ionization of 15,000 or 230,000 particles / cm$^3$ concentration (lots S1L2 and S2L3), with infected injuries 3 days after infection and no ionization (lot S1L5) or infected injuries and subjected negative air ionization 15,000 particles / cm$^3$ (lot S1L6). Of the experimental animals with infected injuries and inflammatory developed process (after 10 days of inflammation) (lot S2L10) and those with infected and uninjured injuries subjected to negative air ionization of about 230,000 particles / cm$^3$ (lot S2L7) or with infected and uninjured injuries subjected to positive air ionization of about 230,000 / cm$^3$ concentration, were observed significantly reduced values or a declining trend of Killing Effect.

The evaluation of oxygen-dependent bactericidal function of PMN granulocytes (oxidative metabolism of phagocytes) investigated by NBT test (tetrazolium test-nitroso-Blau) - the concentration of positive formasan cells from white Wistar rats blood of different experimental groups is shown in figures 4 and 5.

According to available data, the relative (%) and absolute (NX10 * 9 / l) values of the PMN formasan positive cells number from NBT test on WR in the experiment varied depending on the presence of inflammatory and infectious process and on the air ionization applied value, but were mostly high.

Significantly high values, both relative (%) and absolute (NX10 * 9 / L) were found at WR from group 3 with injuries of 3, 0 cm$^2$, infected but unionised (lot S1L5), with infected injuries of 16.3 cm$^2$ and 20.1 cm$^2$ (evaluated at 10 days after infection) but unionized (S2L9 and S2L10 lots).we also should note that on laboratory animals from these groups with different surface injuries, infected but unionized (S2L10, S2L9 and S1L5 lots) has been found the highest concentration of positive formasan PMN cells (P <0.05).

Of experimental WR the presence of injuries with different surface infected but subject to negative air ionization of 15,000 or 300,000 particles / cm$^3$ (lots S1L6 and S2 L7) and those with infected injuries subject to positive air ionization (S3L8) was observed an improvement (only a slight tendency to increase) of relative (%) and absolute (NX10 * 9 / l) number of formasan positive PMN cells compared with controls and significantly lower compared to animals from lots with injury and infection but unionized (S2L10 and S2L9 lots). Similarly, insignificant increased were concentrations values of formasan positive cells of animals without injury and without infection, subject to negative air ionization of 15,000 particles / cm$^3$ (lot S1L2); application of negative air ionization of 230,000 particles / cm$^3$ was mentioned by a substantial decrease of formasan positive PMN cells in the blood (P <0.05).

Of experimental animals with uninflamed injuries subject to positive air ionization of 230,000 particles / cm$^3$ (lot S3L4) were not observed significant changes in the concentration of formasan positive PMN cells and those with inflamed injuries (lot S3L8) - was found a tendency to increase both the relative (%) as well as the absolutely (NX10 * 9 / l) number of formasan positive cells in the blood.

To characterize the immune status has a particular significance the assessment of relative (%) and absolute (NX10 * 9 / l) concentration of lymphocyte populations and subpopulations and their activation properties (blast transformation) under the action of mitogen.

It is noteworthy that on investigated WR were not found significant variations of relative concentration (%) of T-lymphocytes in the blood, the existing deviations integrating in the standard values of deviation.

Figure 6 presents the results of concentration T lymphocytes evaluation (after test E-RFC/CLFR) in absolute values (NX10 * 9 / L). It is of interest the WR with skin inflamed injuries, without air ionization (lot S2L9), has been a significant increase of T-lymphocyte cells expressed in absolute value (5.31 + / -0.62 x10 * 9 / L, P <0.05> 0.01), which may be due to the development of an acute infectious-inflammatory process and the immune response to infection.
Figure 1.

Concentrația (%) de celule fagocitare PMN în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori medii</th>
<th>Deviație standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>81</td>
<td>2.2</td>
</tr>
<tr>
<td>S1.L2</td>
<td>83</td>
<td>3.5</td>
</tr>
<tr>
<td>S2.L3</td>
<td>82</td>
<td>2.1</td>
</tr>
<tr>
<td>S1.L5</td>
<td>76</td>
<td>3.4</td>
</tr>
<tr>
<td>S2.L9</td>
<td>77</td>
<td>2.5</td>
</tr>
<tr>
<td>S2.L10.</td>
<td>75</td>
<td>1.6</td>
</tr>
<tr>
<td>S1.L6</td>
<td>79</td>
<td>1.4</td>
</tr>
<tr>
<td>S2.L7</td>
<td>79</td>
<td>3.8</td>
</tr>
<tr>
<td>S3.L4</td>
<td>63</td>
<td>2.6</td>
</tr>
<tr>
<td>S3.L8</td>
<td>62</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Figure 2.

Concentrația de celule fagocitare (nx10^9/l) PMN în sânge al șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori medii</th>
<th>Deviație standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>2.22</td>
<td>0.167</td>
</tr>
<tr>
<td>S1.L2</td>
<td>2.68</td>
<td>0.432</td>
</tr>
<tr>
<td>S2.L3</td>
<td>1.42</td>
<td>0.306</td>
</tr>
<tr>
<td>S1.L5</td>
<td>2.53</td>
<td>0.243</td>
</tr>
<tr>
<td>S2.L9</td>
<td>2.69</td>
<td>0.378</td>
</tr>
<tr>
<td>S2.L10</td>
<td>2.10</td>
<td>0.203</td>
</tr>
<tr>
<td>S1.L6</td>
<td>1.42</td>
<td>0.352</td>
</tr>
<tr>
<td>S2.L7</td>
<td>1.22</td>
<td>0.339</td>
</tr>
<tr>
<td>S3.L4</td>
<td>1.64</td>
<td>0.121</td>
</tr>
<tr>
<td>S3.L8</td>
<td>1.22</td>
<td>0.232</td>
</tr>
</tbody>
</table>

Figure 3.

Efectul Killing asupra celulelor microbiene (%) din fagocitele PMN sangvine la șobolanii albi Wistar sub acțiunea aeroionizării artificiale

<table>
<thead>
<tr>
<th>Lot</th>
<th>Valori medii</th>
<th>Deviație standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2.L1</td>
<td>77.4</td>
<td>1.06</td>
</tr>
<tr>
<td>S1.L2</td>
<td>79.9</td>
<td>2.26</td>
</tr>
<tr>
<td>S2.L3</td>
<td>71.1</td>
<td>1.18</td>
</tr>
<tr>
<td>S1.L5</td>
<td>71.2</td>
<td>2.27</td>
</tr>
<tr>
<td>S2.L9</td>
<td>70.7</td>
<td>5.50</td>
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<tr>
<td>S2.L10.</td>
<td>68.9</td>
<td>1.86</td>
</tr>
<tr>
<td>S1.L6</td>
<td>74.0</td>
<td>1.32</td>
</tr>
<tr>
<td>S2.L7</td>
<td>68.8</td>
<td>1.16</td>
</tr>
<tr>
<td>S3.L4</td>
<td>61.4</td>
<td>3.59</td>
</tr>
<tr>
<td>S3.L8</td>
<td>59.0</td>
<td>3.74</td>
</tr>
</tbody>
</table>
Concentrația (%): de celule PMN formazan pozitive sangvine în testul NBT la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

![Graph 4]

Valori medii: 7 9 8 11 13 17 10 14 7 10
Deviație standard: 1.0 1.1 1.1 1.0 1.4 0.9 0.9 2.4 2.4 2.8

Concentrația (nx10^9/l): de celule PMN formazan pozitive sangvine în testul NBT la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

![Graph 5]

Valori medii: 0.17 0.24 0.12 0.29 0.34 0.36 0.23 0.19 0.12 0.22
Deviație standard: 0.020 0.050 0.023 0.047 0.060 0.046 0.058 0.031 0.047 0.086

Concentrația absolută (nx10^9/l sau nx10^6/ml): de limfocite T (testul E-RFC) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

![Graph 6]

Valori medii: 4.10 4.08 2.92 4.29 5.31 4.12 3.50 3.22 2.47 2.99
Deviație standard: 0.526 0.467 0.459 0.288 0.617 0.250 0.201 0.406 0.402 0.451
Of WR with no injuries and those with inflamed injuries from lots subject to negative air ionization of about 230,000 particles / cm³ (lots S2L3 and S2L7) and subject to the same maximal values of positive ionization (lot S3L8) where observed some trends of decreasing absolute concentrations of T lymphocytes.

The analysis of data obtained showed that on the animals from control and experimental lot results were not found significant changes in their relative concentration (%) of T-helper lymphocytes (E-RFC/CLFR, 29 °C), being observed some changes of absolute concentrations (NX10 * 9 / L) of these cells.

Figure 7 presents the T-helper lymphocytes concentration of investigated WR.

Thus, a significant increase of the absolute number of lymphocytes T-helper took place at WR with inflamed skin injuries, unionised (lot S2L9); a significant decrease - on animals subjected to maximum positive air ionization of about 230,000 particles / cm³ (lot S3L8 ) (P <0.05) and the tendency of decreasing the absolute concentration of these blood cells – of animals without injury and those with inflamed injuries from lots subject to negative air ionization of about 230,000 particles / cm³ (lots S2L3 and S2L7).

Figures 8 and 10 present the results of T-suppressor lymphocytes concentrations in the blood (T lymphocytes - T lymphocytes E-RFC/CLFR test 29 °C) from experimental WR.

According to these data just for WR lots with uninflamed injuries and subjected to positive air ionization of about was found a tendency of decreasing of relative concentration (%) of T-suppressor lymphocytes. Of animals with or without injuries, infected or uninfected, unionized or subjected to negative air ionization of 15,000 or 230,000 / cm³ were not mentioned significant changes.

Of WR with skin inflamed injuries, unionized (lot S2L9) was found an increasing trend of absolute number (NX10 * 9 / L) of T-suppressor lymphocytes, of animals subjected to maximum positive air ionization of 230,000 particles / cm³ (lot S3L8 ) - was found a significantly decrease (P> 0.05 <0.1) and of animals without injuries and with inflamed injuries from lots subject to negative air ionization of about 230,000 particles / cm³ (lots S2L3 and S2L7) – the tendency of decreasing the absolute concentration of these blood cells.

Mathematical ratio (ratio index subpopulations cell) "T-helper lymphocytes / suppressor T-lymphocytes" has changed slightly, suggesting a prevalence of T-helper cell concentration on the concentration of T-suppressor lymphocytes.

Concentrations of T-cytotoxic lymphocyte cell expressed in absolute values (NX10 * 9 / L) were raised at WR with infected injuries unionized (lot S2L9) and those with infected injuries subjected to positive air ionization of 230,000 particles / cm³ (P> 0.05 <0.1); the trend of decreasing of absolute number of these blood cells was observed on animals without injuries and uninfected, and those with infected injuries and subject to negative air ionization of 230,000 particles / cm³.

Concentrations of T-cytotoxic lymphocyte cell expressed in absolute values (NX10 * 9 / L) were high at WR with infected injuries unionized (lot S2L9) and those with infected injuries subjected to positive air ionization of 230,000 particles / cm³ (P> 0.05 <0.1); the trend of decreasing the absolute number of these blood cells was observed on animals without injuries and uninfected, and those with infected injuries subject to negative air ionization 230,000 particles / cm³. The activation value of T-lymphocyte cells in vitro under the action of a mitogen are the functional property of these experimental WR lots. Figures 13 and 14 preseny the results of the lymphocyte transformation test in blasts (TTLB) cells in vitro activation of lymphocytes with phytohaemagglutinin.

From these data it was found that both the relative (%) and the absolute (NX10 * 9 / L) concentration values of lymphocytes transformer blast were reduced in the blood of WR with infected injuries subjected to negative air ionization of about 230,000 particles / cm³ (lot S2L7) and those with uninfected injury (lot S3L4) and those with infected injury subjected to positive air ionization of about 230,000 particles / cm³ (lot S3L8).

He was mentioned the tendency of decreasing relative number (%) of transformer blast cells on laboratory animals with infected injuries and infectious-inflammatory process lasting 10 days (lot S2L10) and absolute number (NX10 * 9 / L ) of
lymphoblasts from animals subjected to negative air ionization of about 230,000 particles / cm³. It also noted that of animals with intact injuries (lot S2L9) and inflammatory acute infectious process (lot S2L9) number of transformer blast cell in blood showed an increasing trend.

Figure 7.

Figure 8.

Figure 9.
Valoarea raportului subpopulațiilor limfocitelor T-helperi / T-supresori (E-RFC 29°C / E-RFC - E-RFC 29°C) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

Valori medii: 1.42, 1.39, 1.42, 1.55, 1.33, 1.41, 1.36, 1.55, 1.90
Deviatie standard: 0.083, 0.114, 0.083, 0.209, 0.082, 0.076, 0.102, 0.439, 0.208

Concentrația (%) de limfocite T-termostabile (citotoxice) (testul E-RFC, 45°C) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

Valori medii: 10, 9, 10, 101, 11, 21, 0, 9, 16, 17
Deviatie standard: 1.0, 1.4, 1.0, 1.2, 1.1, 1.5, 1.0, 1.3, 1.6, 1.3

Concentrația absolută (nx10⁹/l sau nx10⁶/ml) a limfocitelor T-termostabile (citotoxice) (testul E-RFC, 45°C) în sânge la șobolanii albi Wistar din diferite loturi sub acțiunea aeroionizării artificiale

Valori medii: 0.43, 0.38, 0.37, 0.42, 0.59, 0.51, 0.36, 0.29, 0.38, 0.54
Deviatie standard: 0.076, 0.044, 0.063, 0.065, 0.047, 0.073, 0.035, 0.067, 0.059, 0.079
Concentrația (%) de limfocite T blasttransformatoare (activate la contactul cu mitogenul fitohemaglutinină) (testul de transformare blastică a limfocitelor sangvine in vitro) la șobolanii albi Wistar sub acțiunea aeroionizării artificiale

Valori medii

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<tbody>
<tr>
<td>81</td>
<td>80</td>
<td>81</td>
<td>80</td>
<td>77</td>
<td>73</td>
<td>81</td>
<td>67</td>
<td>65</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Deviație standard</td>
<td>1.6</td>
<td>1.1</td>
<td>1.6</td>
<td>1.7</td>
<td>3.1</td>
<td>1.9</td>
<td>1.4</td>
<td>2.0</td>
<td>2.2</td>
<td>1.5</td>
</tr>
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</table>

Concentrația absolută (nx10^9/l sau nx10^6/ml) de limfocite T blasttransformatoare în sânge (testul de transformare blastică a limfocitelor la contactul cu mitogenul hemaglutinină în vitro) la șobolanii albi Wistar sub acțiunea aeroionizării artificiale

Valori medii

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<tbody>
<tr>
<td>4.89</td>
<td>4.62</td>
<td>3.48</td>
<td>5.14</td>
<td>5.91</td>
<td>4.45</td>
<td>4.16</td>
<td>3.31</td>
<td>2.46</td>
<td>3.19</td>
<td></td>
</tr>
<tr>
<td>Deviație standard</td>
<td>0.567</td>
<td>0.430</td>
<td>0.475</td>
<td>0.293</td>
<td>0.702</td>
<td>0.372</td>
<td>0.257</td>
<td>0.378</td>
<td>0.422</td>
<td>0.469</td>
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</table>
4. DISCUSSION AND CONCLUSIONS

Analysis of the presented results highlights the effect of negative values of air ionization of about 15,000 particles / cm$^3$ and 230,000 particles / cm$^3$ on nonspecific factors of body resistance of white Wistar rats (without inflammatory infectious skin process, induced experimentally on investigated WR lots).

Of experimental WR subjected to negative air ionization of 15,000 particles / cm$^3$ was observed a stimulating tendency of some components of the phagocytosis process including a increase of the absolute number of phagocytic PMN.

Under the action of negative air ionization of 230,000 particles / cm$^3$ concentration at WR were found significantly decreased of the number values of phagocytic PMN, which indicate the suppression effect of high air ions concentration. Is interesting to note that both the action of concentration of 15,000 particles / cm$^3$ and 230,000 particles / cm$^3$ at lots of animals without infectious-inflammatory effect was observed high value of Killing effect of phagocytic PMN on phagocytized microorganisms. Under the action of 230,000/cm$^3$ particles concentration, on experimental laboratory animals was observed and a substantial decrease in the number of positive formasan PMN cells in the blood compared with controls and other experimental lots. These data indicate a stimulation of bactericidal effect of phagocytes (phagocyte concentration is reduced) and oxygen-dependent bactericidal function of these.

Based on the analysis results we should note the role of nonspecific factors of resistance against infection in the acute infectious-inflammatory skin process (over 3 days) and longer (after 10 days) achieved experimentally on WR.

Thus, with the development of infectious-inflammatory process at WR with infected injuries but unionized was observed the tendency of decreasing relative number of phagocytic PMN and an increase in absolute value which is due to high concentration (NX10 * 9 / L) of leukocytes in blood - response to infection and acute inflammation.

Although the total (absolute) of phagocytes number didn’t decrease (has been found just the tendency to decrease of relative value of these PMN cells as has been noted) in the first three days on animals with infected injuries and those with inflammatory-developed process, (after 10 days of infection) was highlighted the decreasing and the trend of decreasing of microorganisms phagocytized number in cytoplasm of PMN phagocytosis, which involves damage of microorganisms properties by that phagocytic blood cells.

It is significat that at 3 days after injuries infection was found a Killing effect stimulation and after 10 days its reduction and a substantial increase of the number of formasan positive cells (the NBT test), which confirming the minimize of the "destruction" effect of phagocytized microorganisms with extension of infectious-inflammatory process

The phagocytosed and nitroso-blau-tetrazolium (NBT) test analysis at experimental WR, indicates the presence of laboratory experimental animals with infected injuries and induced infectious inflammatory skin process a nonspecific defense response of body against infection during the acute phase (3 days) and phagocytic properties and the "destruction" minimizing of phagocytized microoorganism and oxygen-dependent bactericidal function of PMN granulocytes, particularly during the longer-lasting inflammatory process (after 10 days infection / inflammation of skin injuries).

Applying of negative air ionization of 15,000 particles / cm$^3$ concentration on laboratory animals with skin injuries Staph.aureus infected didn’t significantly changed the relative (%) and absolute (NX10 * 9 / L) concentration of phagocytic PMN cells and the number of phagocytized microorganisms has stimulated considerable Killing effect of PMN phagocytic cells on phagocytized microorganisms. Relative and absolute number of PMN formasan positive cells had a growth trend and is therefore slightly lower their oxygen-dependent bactericidal function, which may be due to the presence of injuries and inflammatory infectious skin process

Under the negative air ionization of 230,000 particles / cm$^3$ concentration, on animals infected with Staph. aureus were not found significant changes in relative concentration (%) of phagocytic PMN cells and was observed the trend of decreasing of them absolute number (NX10 * 9 / L) , reducing the Killing effect of phagocytes, and tendency to increase of relative and absolute of formasan positive PMN cells.

These results indicate the possibility of significant damage (decrease) of the phagocytosis process on WR infected and inflamed injuries subjected to negative air ionization with very high concentrations (about 230 000 particles / cm$^3$).

Of the experimental animals in presence of injuries with different surface infected but subject to negative air ionization of 15,000 or 300,000 particles / cm$^3$ (lots S1L6 and S2 L7) and those with infected injuries subject to positive air ionization (S3L8) was observed an improvement trend (a slight tendency to increase) of the relative (%) and absolute (NX10 * 9 / L) number for PMN formasan positive cells compared with controls and significantly lower compared with animals from...
lots with injury and infection but unionized (lots S2L10 and S2L9).

Regarding to the positive air ionization action, it is significant to note that under the action of concentration of 230,000 particles / cm³ at WR in the experimental lots that was found a suppression effect on the phagocytosis process, which was manifested by decreasing the number of phagocytic PMN cells in the blood of laboratory animals with infected injuries, and significantly reduce of the Killing effect. of laboratory animals with inflamed injuries have mentioned the tendency of suppression oxygen-dependent bactericidal function of PMN blood cells.

At WR investigated were not found significant variations of (% T lymphocytes (E-RFC-CLFR), the lymphocyte subpopulations - T-helper lymphocytes (E-RFC-CLFR 29 º C), T-suppressor lymphocytes (E-RFC - 29 º C E-RFC) relative concentration in the blood, existing deviations integrates in the standard values. In the presence of infectious-inflammatory process (the infected and inflamed injuries) in the acute phase has been mentioned a significant increase of T-lymphocyte absolute number (NX10 * 9 / L), and of absolute value of T-helper lymphocytes and the tendency to increase the absolute number of T-suppressor lymphocytes.

In WR blood without injuries inflamed subject to negative air ionization about 230,000 particles / cm³ was observed the trend of decreasing T-lymphocyte absolute concentration and of animals without injuries and those with inflamed injuries-the tendency of decreasing the concentration of absolute T-helper and T-suppressor. Under the positive air ionization of about 230,000 particles / cm³ - was observed the trend of decreasing the absolute concentration of T-lymphocytes and a significant decrease in the absolute number of T-helpers and T-suppressor.

Should be noted that the index report of cell subpopulations "T-helper lymphocytes / suppressor T-lymphocytes" wasn’t subjected to significant changes, only a tendency to increase the value on experimental animals subjected to positive air ionization concentration of 230,000 / cm³. Of WR- with or without infected injuries subjected to negative air ionization 15,000 or 230,000 / cm³ particles the relative values of the concentration of cytotoxic T-lymphocytes - (T lymphocyte-thermostat at 45 degrees C) weren’t significantly changed. Under the positive air ionization action of 230,000 particles / cm³ was reported a significant increase of relative and absolute number of cytotoxic lymphocyte cells, being observed the tendency of decreasing the absolute number of these blood cells on animals subjected to negative air ionization of 230,000 particles / cm³.

It is noteworthy that some changes occurring as a result of the activation blastogenesis-T lymphocytes process in vitro. Thus, both the relative (%) and the absolute (NX10 * 9 / L) concentrations values of blast transformer lymphocytes were reduced at animals with infected injuries subjected to negative or positive air ionization of about 230,000 particles / cm³. Reduction of the relative blast transformer cells number has been mentioned at animals with infected injuries and infectious-inflammatory process lasting 10 days, those with injuries inctate acute inflammatory and infectious process is observing an increasing trend of the blast transformer cells number in blood.

The results analysis allow to establish that both tests characterizing some non-specific resistance factors of the animal organism (phagocytosis, NBT test) and those of the lymphocyte immune system that characterizes the immune status were framed in the ionization normal limits with concentrations of about 15,000 particles / cm² of Wistar white rats. Under the action of the same values of negative ionization was found a stimulation of different phases of phagocytosis both on animals without skin injuries and those with infected injuries and acute inflammatory-infected process. So under the action of higher concentrations than natural, it was found that with faster regeneration of the infected and inflamed skin area occurred antibacterial mechanisms activation of blood cells - neutrophils in the PMN phagocytosis (enhanced Killing Effect) and NBT tests.

The results indicate the possibility of (decrease) phagocytosis process damage at Wistar white rats with infected and inflamed injuries subjected to very high concentrations (negative and positive) of negative air ionization (about 230 000 particles / cm³). The T lymphoma system values weren’t significantly changed by negative air ionization of about 15,000 particles / cm³, but on the animals subjected to negative or positive air ionization of about 230,000 particles / cm³ concentration appeared some drop in the concentration of T lymphocytes and T-helpers subpopulations, and decrease of the lymphocytes activation process (blast transformation), suggesting the possibility of suppression of some factors of the immune status to positive and negative maximal concentrations of air ionization.

Thus, from the cellular immunological investigations on the T lymphocytes system have found results that showing the presence of some changes of the lymphoma immune system parameters on experimental animals subjected to negative and positive air ionization of 230,000 particles / cm³concentration.
The data are of interest both from practical and basis for research and indicate the need for studies development in this area.

*Results obtained in phase IV(Responsible– CPII Dr.b. Simionca Iuri), Project Nr.466/2004 – 2007(Project Manager- Conf. Dr. fiz. Enache Liviu)

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LITHIUM MINERAL WATER

Munteanu Constantin¹, Munteanu Diana²
¹ SC BIOSAFETY SRL-D
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Abstract
Hydrological surveys showed that Romania basement contains a variety of balneary resources located within on the surface crust. Mineral waters are spread over more than 20% of the country at different depths, with a wide range of physical, chemical and therapeutic properties depending on their genesis.

Balneary resources are represented mainly by therapeutic minerals that the physicochemical properties answer the needs of medical and prophylactic maintenance, enhancement and restoration of health, work capacity and physical and mental comfort of the individual.

The surface waters arising from a natural source or updated by drilling and whose physical and chemical characteristics that may exert dynamic pharmaco-therapeutic are considered therapeutic mineral waters. Mineral waters are waters that have a variable content of salts, gas, minerals, radioactive elements, which gives them therapeutic properties. In the past, name of mineral water was attributed to all shallow or groundwater mineral water that could be used for therapeutic purposes. In recent years, mineral water that could be used for therapeutic purposes have been given the name of curative water.

Lithium arouses a great scientific interest because, although his structure is so simple, easy to analyze, with chemical and physical properties well established the myriad of the effects on biological systems by influencing many cellular processes and molecular and the mechanism of action are still unclear generates a mystery that modern science attempting to decipher.
In vivo and in vitro studies have shown that lithium exerts multiple effects on receptor signaling mediated by neurotransmitters, ion transport, signaling cascades, hormonal regulation, and diurnal rhythm of gene expression (Cyrus et all, 2006). Unfortunately, the molecular mechanisms responsible for these effects are still a subject of debate. Biochemical mechanisms of action of lithium appear to be multifactorial and interrelated with functioning of several enzymes, hormones and vitamins, as well as factors of growth and transformation (Schrauzer, 2002).

Acute effects of lithium are mediated through inhibition of enzymes involved in two distinct but interactive signal paths - the path of protein C kinase and glycogen synthase 3β kinase cascade - which converge at the level of gene transcription.

The expression of some genes, including transcription factors, is significantly changed by chronic administration of lithium. Chronic lithium treatment increases the neuroprotective bcl2 protein expression, leading to an interesting possibility that some effects of lithium to be mediated through effects neurotrophic / neuroprotective (Ikonomov and Manji, 1999).

In centre of Romania, in the county with the same name, located in the Brasov Depression, at the western foot of the Vrancea mountains at an altitude ranging between 550 and 600m, 31 km east of the city of Saint George, is located Covasna city – an important center for bottling mineral water.

The medicinal mineral water known as Maria water is bottled in Malnas Bai resort. Malnas Bai resort is situated in the gorge that separates the mountains Bodoc from Baraolt Mountains, about 22 km from St. George. Climate has no large thermal amplitudes, the average annual temperature is 70 C and average annual rainfall of 600 mm. The resort formation dates since 1759 and after 1865 and its reputation reached abroad.

Maria mineral water is a water bicarbonate, chloride, sodium, carbonated, hypotonia, used for internal cure and packaging.

Maria medicinal mineral water is bottled since 1904 when it was recommended to treat various digestive disorders such as digestive (chronic gastritis with hyperacidity, gastric and duodenal ulcers, chronic colitis, chronic constipation), shares hepatobiliary (dyskinesia bile, chronic hepatitis, chronic pancreatitis, chronic cholecystitis not calculated or calculated), associated diseases: neurasthenia, migraine, emotional disturbances.

Maria medicinal water from Malnas Bai, containing 8 mg per liter of lithium been applied in clinical and experimental research in the treatment of migraine and affective disorders, diseases that have entered the therapeutic spectrum of water in the past.
Lithium effects on the nervous system have been intensively studied thanks it for use in the treatment of manic-depressive psychosis (Gilles and Bannigan, 1997, Lenox and Hahn, 2000).

Treatment with lithium chloride began from the day 6 of cultivation of glial cells after lag phase, which corresponds to cell multiplication start, to cell islands formation and pronounced cell differentiation.

Treatment with lithium chloride involves using a growing medium prepared with a quantity of lithium chloride corresponding to 1 and 2-mM lithium concentrations. According to the used protocol, the preparation of lithium chloride medium involves obtaining a stock solution of 20 mM lithium chloride in DMEM medium, from which the specific volume is used to obtain the desired concentrations.

Medium change and the treatment application with lithium of 1 and 2 mM concentration, occurs at a frequency of three days of cultivation.

Concentration of 1 mM lithium corresponds to therapeutic serum level of lithium, achieved in the treatment of manic-depression, a level that should be closely monitored so as not to be exceeded.

Concentration of 2 mM lithium is a toxic dose to the body, as demonstrated most of studies conducted till now in various research centers that focused on the effects of lithium.

Biological effects of lithium can be divided into: short-term effects (manifested shortly after application and probably mediated by complex cellular available) and long-term effects (assumed to be based on selective changes of gene expression that occur after a delay of several days to weeks). Many short-term effects of lithium appear to be specific to cells or tissues. Examples include short-term stimulatory effects of corticotropin secretion induced by lithium of rat anterior pituitary cells and a massive release of glutamate of the brain sections treated with lithium. Inhibitory effects of short-term treatment with lithium are evidenced by the secretion of aldosterone induced by angiotensin II adrenal glomerular cells and the rate of relaxation that follows the induction of cholinergic smooth muscle contraction. Inhibitory effects of short-term treatment with lithium are evidenced by the secretion of aldosterone induced by angiotensin II of adrenal glomerular cells and the rate of relaxation that follows the induction of cholinergic smooth muscle contraction.

Among the long-term phenotypic changes may be mentioned changes induced by lithium on the circadian rhythm and of course behavior changes in patients with bipolar disorder appeared after 2-3 days or few weeks.

Chronic lithium treatment on laboratory rats leads to a running persistent deficit at some behavioral tests (active and visual avoidance of the maze), taking into account that the memory deficit in a task space is transient.
Glial cells grown in the presence of lithium-rich mineral waters is the experimental model to verify hypotheses on their role in improving the growth parameters of glial cells in vitro.

Maria mineral water treatment involves the preparation of media for cultivation replacing a part of double distilled water necessary for the process of obtaining with Maria mineral litiniere water. The experimental treatment involves the use of media with 50% and 25% Maria mineral water, which basically means the replacement of 50% and 25% respectively of necessary double distilled water with Maria mineral water from Malnas-Spa.

A third alternative case for tracking the water effects is represent by adding of variant with 25% Maria mineral water to a concentration of 1 mM LiCl to monitor the effect of increasing the total amount of lithium in water.

Sterilization of culture media prepared with Maria mineral water and used for the treatment of glial cell cultures is performed by nitrocellulose filtration membrane with pore diameter of 0.2 mm.

Medium change and the treatment with Maria mineral water of 50%, 25% and 25% + 1 mM LiCl concentration, occurs at a frequency of two to three days of cultivation.

The treatment with Maria mineral water began from the day 6 of cultivation of glial cells after lag phase, which corresponds to the start islands cell formation and to pronounced cellular differentiation.

Choosing water concentration was correlated with the physiological capacity of water ingestion of the organism, under the assumption that in blood may be replaced within 24 hours maximum 25% of serum with water consumed daily (1.5 - 2 liters of water a day), to be filtered and removed the salts through kidney, digestive and skin.

Maria mineral water of 50% concentration is chosen only to experimental pursue to investigate the effect of this high level has on the glial cells in vitro. This level has no therapeutic value, because the body has no way to have a such quantity of consumed water.

From the experimental point of view, the 50% concentration represents a positive control of the effects that Maria mineral water may have on cells.

Preliminary experimental data of our studies have shown that by replacing 100% of double-distilled water needed in the technological process of preparing the medium for the cultivation of glial cells culture, culture destruction occurs in 48-72h after application.

Was found that Maria mineral water can not provide the minimum conditions for survival of glial cells in vitro.
Fig. 39 Microscopy aspect of 15 days of culture treated with MARIA water 25% (A- x 15, B- x 15, C -x 20, D-x 15)

References
Editing regulations

(peer-review protocol)

The manuscripts will be submitted as attachment to the email in Word format (to culturi@gmail.com). Photo processing, scanning, graph processing –if needed-are the responsibility of the editing team. Language of papers is English. Articles can be published with translation into Romanian.

After manuscript receipt, the corresponding author will receive a short e-mail confirming the receipt, which will contain the registration number, the date the manuscript was received and the fact that the manuscript was handed out to the Editorial Board. The Journal Editor chooses 2 peer-reviewers (from the Editorial and Peer-review Board) and sends them by e-mail the manuscript.

The reviewers' decision (approval with no changes, approval with major/minor changes, rejection) will be immediately communicated by e-mail to the corresponding author by the editor.

If the manuscript gets approval with changes, the corresponding author shall send the improved manuscript within 4 weeks. The editor will convey the corresponding author's answer to the peer reviewers. If they are satisfied with the corresponding author's answer, they will send the subject editor the decision of approval for publication of the improved manuscript.

If the peer reviewers consider that the corresponding author did not meet/or met poorly the revision requests, they will deny the approval for publication, which will be communicated to the editor.

The approval for publication once taken by the reviewers, the decision will be communicated in editorial meeting.