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## THE MODELING OF CERTAIN PROBLEMS OF SPACE BIOLOGY IN EARTH CONDITIONS

G.E. KHACHATRYAN<sup>1</sup>, N.V. SIMONYAN<sup>1</sup>, N.I. MKRTCHYAN<sup>1</sup>,  
V.B. ARAKELYAN<sup>2</sup>, S.SH. TATIKYAN<sup>3</sup>, V.M. TSAKANOV<sup>3</sup>,  
P.M. ANTONYAN<sup>4</sup>, S.K. KARAMYAN<sup>4</sup>, V.V. HARUTYUNYAN<sup>1</sup>

<sup>1</sup>A.I. Alikhanyan National Science Laboratory, <sup>2</sup>Yerevan State University,

<sup>3</sup>CANDLE Synchrotron Research Institute, <sup>4</sup>National Center of Oncology of MH RA  
garnik@mail.yerphi.am

The paper represents some data obtained in recent years in the field of space microbiology while conducting research in conditions simulating cosmic space, as well as directly on the spacecrafts flying in near-Earth orbits. The possibility of conducting research on this topic in Armenia by efforts of a number of research teams and their preliminary achievements in related fields are reviewed.

*Space biology – extreme conditions – ionizing radiation – electron beams –  
proton beams of 18 MeV – low temperature*

Հոդվածում ներկայացված են վերջին տարիներին տիեզերական մանրէաբանության ոլորտում կատարված հետազոտությունների արդյունքները, որոնք իրականացվել են ինչպես տիեզերական տարածքը մոդելավորող պայմաններում, այնպես էլ մերձերկրյա տարածքում թռչող սարքերում: Քննարկվում են Հայաստանի մի շարք գիտական կոլեկտիվներում այդ ասպարեզում հետազոտություններ կատարելու հնարավորությունները և հարակից ասպարեզներում նախնական ձեռքբերումները:

*Տիեզերական կենսաբանություն – էքստրեմալ պայմաններ – իոնիզացիոն ճառագայթներ –  
էլեկտրոնային փնջեր – 18 ՄեՎ պրոտոնային փնջեր – գերցածր ջերմաստիճաններ*

В работе приведены некоторые данные последних лет, полученные в области космической микробиологии при проведении исследований в условиях, моделирующих космическое пространство, а также непосредственно на аппаратах, летающих на околоземных орбитах. Рассмотрены возможности выполнения исследований по этой теме в Армении силами ряда научных коллективов и их предварительные достижения в смежных областях.

*Космическая биология – экстремальные условия – ионизирующее излучение –  
электронные пучки – пучки протонов 18 МэВ – сверхнизкие температуры*

In recent years, after a rather long break, an abrupt interest arose in the world related to space biology again. It is caused by various resurgent projects of sending expeditions to other planets of the Solar System, particularly to Mars, as well as by search for arguments in favor of extraterrestrial life – the Panspermia hypothesis. The details of biological experiments obtained in the 80s of the last century, during Soviet and American spacecraft flights, are processed and reviewed. Biological researches are conducted on board of modern spacecrafts and in ground-based laboratories, in which the

conditions on the surface of Mars and Venus are modeled and analyzed [9, 20, 31, 32].

It is important to emphasize that until recently mainly the influence of separate stress-forming extreme factors on living organisms, particularly on microorganisms, has been studied. A classic review of the study related to microorganisms' capability to live under the influence of some extreme factors separately may be considered the fundamental group work of a number of authors, under the general edition of D. Kushner, "Microbial life in extreme environments", published in 1978 [6]. It presents a detailed result of various aspect influence on microorganisms study in key stressful conditions existing on Earth and near-Earth regions: low (up to  $-40^{\circ}\text{C}$  and lower) and high ( $80-90^{\circ}\text{C}$  and higher) temperatures, high (up to 1000 atmosphere and higher) pressures, extreme values of pH, high concentrations of different salts, heavy metal high concentration presence, and, finally, life under irradiation by non-ionizing and ionizing radiations.

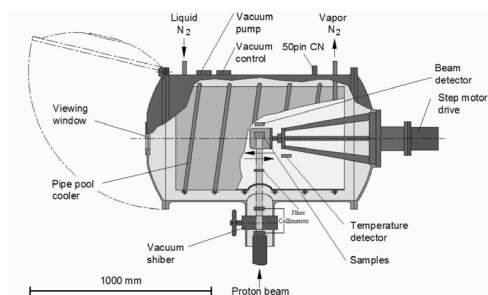
In recent years result studies are being carried out for the simultaneous effects of these factor complexes. It should be noted that despite of certain difficulties there is a close collaboration among Russia, EEC countries, USA, Japan, South Korea when performing experiments in cosmic space environments being particularly evidenced by a recent "Bion-1M" craft flight, the greater part of the researches carried out on it were devoted to the experiments related to space biology [20, 21, 32, 33]. An important focus in these studies was given to the works comprising the behavior of different microorganisms in extreme conditions. And it is quite natural. In order to understand the complex, multiple-factor processes a method of analysis has already been developed, resulting in the investigated object or process simplification. Actually, a cell cannot be taken as a simple formation, however, the unicellular microorganisms, in comparison to multicellular ones, have a number of qualities that make them an excellent model object. Firstly, the short generation time. The rapid change of generations allows identifying the effect consequences of the stressful factors under study in a short time and gain statistics. Secondly, it allows harvesting large amounts of target biomass in a short time, which is more convenient to work with. Thirdly, it is more comfortable to perform various manipulations and experiments with small-size objects.

It can be claimed that science is still on the threshold of solving many problems of space biology, the main of which being the spread of organic material and of life itself throughout the universe, and the survival: identification of the environment in which it is possible for the living organisms to be adapted (to this or that extent) to space conditions. This is evidenced both by the nature of incoming information and by publication format. Very often new information is represented in the form of reports on conferences, interviews and briefings by the leading experts of this branch [4, 20, 21, 27, 31]. Two aspects should be distinguished in this case. The first aspect is related to the travel in a spacecraft through the cosmic space, for example, for a flight to Mars. In this case the analysis of the conditions is touched upon under which living organisms safely get from one location of space to another one with a minimal damage to their life, and viability preservation over a sufficient time after the stressor exposure completion. These issues are partially solved by experiments carried out on near-Earth space stations.

However, it should be noted that near-Earth space stations like "Mir" fly at a height of only 300 km and, actually, are still within Earth atmosphere (the beginning of exosphere boundary is at a height of about 1000 km [11]), and under the protection of Earth magnetic field, extending from 60 to 100 thousands km according to various data. A more difficult task is to stay in deep space, where, as it is known, the radiation fields in cosmic space are characterized by a broad charge composition of the electrons and protons and uranium nuclei, with an energy range from tens of eV to 1018-1019 eV and the range of linear energy transfer (LET), determining the character of the local effect on

the cells and tissues of biological objects, from tenths of keV/mcm up to 103 keV/mcm [12]. Such problems require protection condition simulation which would not complicate the spacecraft output process beyond the Earth because of its heavy weight on the one hand, but would be sufficient to ensure the survival of biological objects on board of such a device for a long flight time (years) on the other hand. The second aspect is related to the transfer of biological material through the cosmic space on any "space carrier" (meteorites, comets and asteroids [13, 26, 28, 34, 35]), and the solution of some tasks on clarification of the following question: to what extent the supposed "space carriers" can contribute to such material transference (being a protection for it), and to what extent this material can be damaged in the course of such a "journey".

Such problems can be solved in the Department of Applied Physical Researches (Applied R & D Division) of A. I. Alikhanyan National Science Laboratory (Yerevan Physics Institute) (ANL-YerPhI), where a device called "measuring vacuum chamber" has already been operating for several years. It enables to simulate cosmic space conditions. In particular, to study the effects of combined exposure on solids of low-temperature, vacuum, and electron beams of varying intensity. The temperature in this chamber may be increased up to 100 °K and even lower, a vacuum of the order  $10^{-3}$ - $10^{-4}$  torr can be achieved. The chamber provided with a special window that is connected to a beam transporting channel, by which the test samples may be exposed (fig. 1).



**Fig. 1.** The measuring vacuum chamber and communications scheme, ensuring required parameters' formation.

Thus, the basic space simulating set of conditions is provided, except for, of course, weightlessness. It should also be noted that it is possible to improve its technical parameters, if necessary, by approximating the simulation conditions closer to the "space" ones. It is assumed that a sample chamber can be installed in the experimental C-18 cyclotron hall (ANL-YerPhI), which will allow carrying out similar researches with proton beams of energies up to 18 MeV. This area of proton energy is of particular interest in biological experiments.

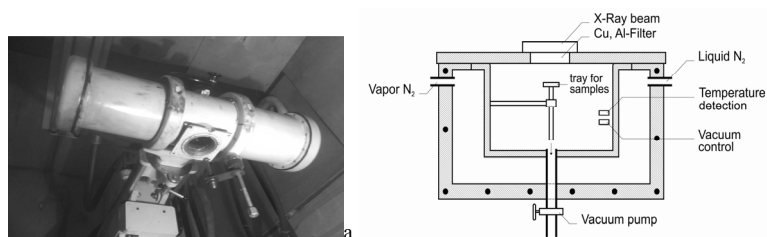
The main purpose of such researches is finding out the chances of terrestrial bacteria survival during their incubation in conditions maximum similar to space, and the detection of the consequences of experimental conditions prolonged exposure on them. There is evidence that in near space conditions, for example, various microorganisms, identified as *B. licheniformis*, *B. subtilis*, *B. sphaericus* and *B. pumilus* are detected on the outside surface of the ISS. However, at the same time it should be emphasized that they were found "in areas with diagnosed surface pollution, possibly serving as nutrient and UV microorganism preservation media or providing "linkage" to station surface [4]. Being based on the results of a cycle of experiments carried out on ISS the Roscosmos specialists (FSUE CSRIMB) noted that "the data obtained on the ISS surface on the chemical and biological composition of cosmic dust allows us to suppose

the existence of "ionospheric lift" mechanism which carries out the transfer of tropospheric aerosol from the ground to the top of the ionosphere". This was confirmed by another discovery: "In areas of the VL2 MRM2 window construction, being located upstream, the fragments of DNA of Micobacteria (heterotrophic marine bacterial plankton dwelling in the Barents Sea) and the DNA of extremophilic bacteria Delftia genus were revealed in 2013. A number of facts are obtained confirming that a significant marine bacterial mass transfer to the ISS orbits is possible" [4].

It is obvious that experiments carried out in simulated conditions with variation of these factors will also provide valuable information on the chances of unicellular organisms' survival and an identification of prolonged exposure effects of these factors. This requires having organisms with clearly defined characteristics, changes of which can be easily identified. Such kind of an issue can be solved by carrying out a series of experiments on biological materials (aerobic bacteria, bacilli, facultative anaerobes), that has recently been studied at the Division of Applied R & D Division (ANL-YerPhI) by the group of Radiation biophysics. These microorganisms are selected based on their high tolerance to high concentrations of certain xenobiotics, and as it turned out, some of them possess a number of physiological and biochemical properties being quite suitable as a criteria to be used in these studies. Up to date the influence of insecticide actara on a number of cultures is most studied in details [23]. In particular, these cultures show the selective ability to grow on various nutrient media at actara concentration variation. The ability of some of them to subject a number of xenobiotics to biotransformation is of great interest as well [10, 16, 22].

The authors of this article are inclined to a long-expressed point of view that the comets are particularly promising as carriers, which are supposed to represent a set of a large amount of water ice, stones and "dirt". On the one hand such kinds of carrier are able to contribute to the biological material conservation and on the other hand they can serve as a sufficient protection against extreme factors' damaging effects and result mitigation of their impact. The actuality of this supposition in favor of comets for the scientific community is evidenced by the decision of the European Space Agency to send a special research probe Rosetta to the comet Churyumov-Gerasimenko, and the attempt to land the descending module Philae on its core. Therefore, different combinations of possible comet components (ice, gravel, sand, porous materials such as perlite and diatomite, silica gels and so on) are proposed to be taken as "space carrier" model.

The usage of porous materials can, in particular, show: to what extent the cellular material "immobilization" in the pores of the carrier may be a positive factor for survival of microorganisms in such extreme situations. Thus, it is well known that the lifetime of immobilized proteins, enzymes, some subcellular structures, and the cells themselves is far increased. At the same time the negative effects of unfavorable factors (high temperature, ionizing radiation) are reduced [3]. It is supposed to evaluate the survival of these microorganisms by the plotting of factor-effect curves and registration of possible changes in their phenotypic, morpho-physiological and biochemical characteristics after exposure to different combinations of extreme factors.



**Fig.2.** a – the general view of the radiating head of RUM-17; b – the scheme of top box for sample irradiation.

For this purpose it is supposed to freeze aqueous suspensions of bacteria (aerobic and facultative anaerobes) in an ice chamber in the form of a small core or bead, the size of which (about 2 cm in diameter) would be correlated to the one of the ionizing radiation beam (about 3 cm). The temperature of the manufactured ice core/bead is supposed to reduce further using liquid nitrogen. The target will be placed on a special tray in the chamber. As it was indicated above, various excipients are supposed to be used, namely: perlites, diatomites, silicagels, relatively small and large sand shingle. The duration of low temperature, vacuum, radiation exposure will vary. It is also planned to use different quality radiation, in particular, there is a source of X-rays – the unit RUM-17, at the researchers' disposal and a device similar to that shown in fig. 1 is supposed to be constructed (see. fig. 2 a, b). The target absorbed doses and beam intensities will also vary. The results will be compared with the following controls: the initial cellular materials and cells packaged into the core/bead but not subjected to vacuum and ionizing radiation, but only to low temperatures. It is as well supposed to carry out a comparison of complex factor impact results with the one of individual stress-forming factors.

Note that in the near future it will be able to conduct researches with proton beams using Cyclone-18 (with beam energy up to 18 MeV) in ANL (YerPhI). It is known that ionizing radiation is an effective source of mutations. The possibility of mutant cells obtaining, being of special interest from a practical point of view, is another area of research of this proposal having a practical consequence. From this point of view electron, proton and various accelerated ion beams are of special interest in radiobiology. The possibility of proton beam usage on Cyclone-18 is a unique opportunity to study the bacteria mutagenesis process. It is known that the efficiency of mutagenesis increases with heightening of relative biological effectiveness (RBE) of beams applied. RBE increases with linear energy transfer (LET) value increasing, which takes place at relatively low energies of particle.

There is very insufficient data in the literature on the biological effects of low-energy protons. Usually the range of energies from 25 MeV or higher was considered. This was primarily dictated by the practical needs of radiation therapy, which uses proton beams of 400 MeV and higher. To achieve the desired values of energies it is necessary to use braking devices, which leads to beam energy scattering (dissipation). The use of Cyclon-18 will allow to perform biological object irradiation under direct proton beam, at the same time the efficiency will be determined by two factors: a sufficient proton particle path length in water equivalent medium and high value of LET (about 5 KeV/mcm), resulting to a significant biological medium ionization. The data below presents the yield of mutant cells for the accelerated ion beams in the bacteria *E. coli*, which are often used in model experiments [8]. Note, that the proton irradiation with 25 MeV energy leads to significantly large changes of cells (the duration of individual phases of the mitotic cycle) than when exposed to protons of higher energies [17].

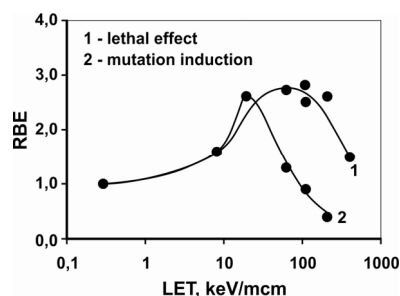


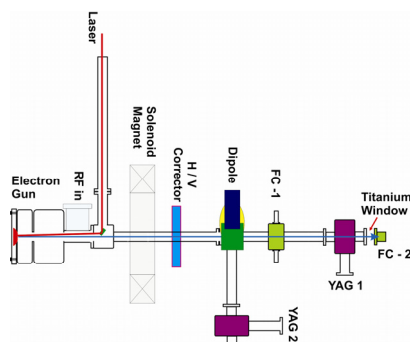
Fig.3. The dependence of RBE on LET particles based on lac- mutations induction criterion and bacteria *E. coli* lethal effects (from E.A. Krasavin work [8]).

With the decrease of proton energy a significant shift of cytogenetic and other irregularities occur towards biological effect worsening mainly associated with an increase of LET. At a value of 2 MeV LET reaches 17 keV/mcm [17].

The RBE of mutation induction, when irradiated by accelerated ions, reaches a plateau, and is rapidly approaching the maximum of lethal terminations for *E. coli* cells (fig 3). It is expected that the use of proton beam energy of 10 to 18 MeV will allow avoiding any ambiguity caused by the dissipation because of the need to inhibit the beams with higher energies, to achieve the desired ones in the target area. Thus, by conducting a purely research work we will have a chance to solve a practical problem as well: to obtain microorganism cultures with increased target activity.

The group of Radiation biophysics at ANL (YerPhI) has the corresponding experience for studying the effect of ionizing and non-ionizing radiation of different quality (X-rays, gamma rays, electron beams, alpha particles, accelerated carbon ions, as well as UV and laser radiation) on microorganisms of various genesis (yeasts, bacteria, bacilli) [1, 14, 15]. In addition, the laboratory conducted a study of the simultaneous exposure of different quality radiation on cells, and mechanisms of a combined radiation action, leading to a particular modification of cell viability of different genotypes. It should be noted that some members of the working group collaborated with the Division of Biological Research of JINR in conducting multi-aspect experiments under the general supervision of Professor E.A. Krasavin, on modeling genetic effects of radiation arising during the simulation of cosmic radiation beams by accelerated heavy ions. Also a mechanism defining the distinctions in biological efficiency of radiations, differing on LET, on the cells of a different genotype was investigated. Laboratory staff also participated in the experiments of "Genome" and "Rhythm" projects. In particular, the Yerevan Physics Institute conducted radiation experiments on *E. coli* strains of different repair genotypes by electrons and X-rays in different experimental conditions and at JINR with the same strains on heavy ion accelerator U-200 of the Laboratory of Nuclear Reactions [5, 7]. There is also experience in the study of long-term exposure of low doses on radiosensitive and radioresistant bacteria [2, 18], which can be useful for evaluating the effects of prolonged exposure to low doses of ionizing radiation on bacteria included in the cores and immobilized on porous carriers. The group also has many years of work experience on the immobilization of enzymes and cells [19, 29]. Experiments on cultured cells under fasting conditions were also carried out [24, 25].

It is necessary to note that the ANL (YerPhI) has also additional abilities for biological material irradiation. In addition, it is planned to have a close collaboration with the CANDLE Institute, which has launched a unique in its parameters AREAL accelerator that provides the ability to perform the irradiation process by different quality beams. AREAL – is a 5 MeV electron accelerator with laser driven photocathode RF gun. The schematic layout of AREAL is presented in fig. 5. AREAL is composed of electron gun with copper photocathode, mirror system for high energy UV illuminating laser, solenoid and corrector magnets, dipole magnet with spectrometer arm, diagnostic screen stations for beam profile and energy measurements, two faraday cups: internal and external for beam charge measurements. For the extraction of electron beam from the vacuum pipe two experimental stations equipped with Ti electron windows are used. The layout of the accelerator is flexible and allows a direct measurement of electron beam parameters just before the extraction. Titanium windows are separating the ultra-high vacuum environment 10<sup>-9</sup> torr from the atmosphere. Nevertheless, they are transparent for the electron beam (99.9% transmission) and do not destroy the electron beam profile and energy distribution. External Faraday Cup is used to measure the extracted charge without and with sample, thus precisely estimating the sample absorbed radiation dose. Besides, the experimental measurements for specific samples FLUKA calculations are performed and compared with experimental results.



**Fig.5.** The schematic layout of AREAL facility.

Another advantage of the AREAL facility is the formation of electrons emitted from photocathode by means of illuminating laser. Forming and changing the main parameters of falling laser, such as transverse spot size, laser intensity/energy, pulse length, allows to generate the electron beam with main parameters similar to the laser profile ones. For other types of electron emission to form an electron beam with parameters similar to the one of AREAL additional accelerator devices, such as bunch compressors, velocity bunching cavities, etc., would be required. The main parameters are quite flexible and available to change within a range to pre-define the electron beam quality. Tabl.1 presents the characteristic parameters for AREAL electron beam. In addition to single bunch operation a new multi bunch operating mode is under development.

**Table 1.** Main parameters of electron beam

Parameter	Single bunch	Multi bunch
Beam energy	Up to 5 MeV	Up to 5MeV
Bunch length (FWHM)	0.4 – 9 ps	0.4 ps
Beam diameter at Ti window	2 - 10 mm	2-10 mm
Bunch charge	10-250 pC	10-20 pC
Emittance $\epsilon_{x,y}$ (mm-mrad)	< 0.35	< 0.35
Pulse repetition rate	1-50 Hz	1-50 Hz
Bunches per pulse	1	16

In result of the planned operations the new information concerning the survival of the bacteria which have, in particular, the specific physiological and biochemical properties, during the incubation in above described extreme conditions under the action of the latter in various combinations on microorganisms will be received. The rates of their growth in these conditions, the growth effects, characterizing the degree of reversibility or irreversibility of damaged cells, and their phenotypic, morphological physiological, biochemical and other characteristics will be evaluated. It is also expected to register mutant bacterial cells, including those with enhanced target activity to certain pesticides used as substrates.

It is expedient to add that the results of the proposed works may also be the basis for the solution of some radiobiological problems of medical orientation, in particular, to optimize the treatment procedures in radiotherapy during cancer treatment using the cell cultures received on the basis of tissues obtained during a biopsy for histological analyzes at National Center of Oncology. In particular, the results of this research may contribute to the development of new modes for fractionation and optimization of dose value per fraction during various tissue irradiation with revealed pathology.

All the foregoing gives grounds to assert that the ANL (YerPhI) conditions together with the technical possibilities of CANDLE will allow to perform a complex of similar activities in terrestrial conditions, to model and solve certain problems of interest to space biology.

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