3.1.1. SPACE BIOLOGY AND PHYSIOLOGY

3.1.1.1. Role of support afferentation in control of tonic muscle activity

The results of studies that were performed in space flights (SF), in simulation studies and in animal experiments have shown that the decline of gravitational loading is followed by disturbances of activities of all mechanisms and structures of the motor system, the complex of which composes the patterns of "hypogravitational ataxia syndrome" and of "hypogravitational muscle detraining syndrome".

Analysis of the nature of these alterations reveals the leading role of the supportlessness in their development. It was suggested that the withdrawal of the support in real and simulated weightlessness and the decline of the support afferentation can be a factor that suppress tonic muscle activity under microgravity conditions and thus support afferentation can be considered as a trigger for activation of tonic muscle system.

To test this hypothesis the series of experiments with the mechanical stimulation of the support zones of the soles under conditions of dry immersion (DI) were performed. In dry immersion model subject is separated from the water by waterproof fabric that allows keeping them as necessary under these conditions without any discomforts for many days. Under the DI procedure subjects were placed in the horizontal position into a tub measuring 200×100×100 cm. The tub filled with water that was kept at a constant temperature of 33±0.5 ^{o}C. The subject was separated from the water by the free floating waterproof, fabric and dressed in comfortable training suit. Under this condition the subject was floating in the water in the prone horizontal position for 7 hours or 7 days.

All of the subjects were exposed to 7-hour or 7-days DI twice. In the first series that was served as a control the immersion was not accompanied by any support stimulation. In the second one mechanical stimulation of the soles support areas, i.e. the heel and the forefoot with pressure of 0,5±0,1 kg/cm^2 was applied every day for 20 minutes of every hour during 6 hours a day in regime of walking — 10 minutes of slow pacing (75 steps/min) and 10 minutes of fast one (120 steps/min). Mechanical stimulation in regime of walking was performed using the Compensator of Support Unloading (CSU).

The electromyographic activity, muscle transverse stiffness, force — velocity characteristics and electromyographic and kinematics parameters were analyzed.

The results of study show that support withdrawal is followed by development of significant changes in all parts of the tonic motor system. In full agreement with earlier obtained experimental data transition to hypogravity was followed consistently by the significant decline of force-velocities properties (on 20%) and electromyographic activity level of all extensors under study that was accompanied by the increase of flexor muscles activity. Suppression of the extensors activities developed in DI at once and was maintained unchanged through the whole time of exposure independently of its duration value (7 hours or

7 days). Restoration of the activity was observed only in case of DI interruption or when mechanical stimulation of soles support zones was applied.

It is important to emphasize that the response to the mechanical stimulation of the support zones of the soles carried out all features of systemic reactions. Stimulations of the fore foot zones was followed by clear contractions of ankle extensors. Stimulation of the fore feet zones on the opposite was followed by flexors contraction.

During the first 24 hrs of DI the m. soleus stiffness dropped down sharply to 75 % of initial level and then stayed unchanged. The stiffness of m. tibialis anterior on the opposite showed a deep tendency for an increase during the first 24 hrs of immersion and later slowly decreased.

The study of electromyogtaphic pattern of locomotions has shown after DI in control series the amplitudes of EMG of both of the leg extensors increased significantly. However an increase of the amplitude of m. gastrocnemius was considerably lager than that of m. soleus. Therefore the ratio of m. soleus and m. gastrocnemius activities after immersion decreased — 0.8 in control instead of 0.5 after DI. The amplitude of EMG bursts in m. tibialis anterior after exposure to DI increased on 30–50 %.

Mechanical stimulation of the support zones of the soles under supportless conditions eliminates of all abovementioned effects, including changes of the transverse stiffness and the maximal voluntary forces of postural muscles and decline of participation of postural muscles in locomotor activity.

The timeline of the above changes could differ in many features, though the order of their appearance in all subjects was unchanged. Foremost, with latency period 3–6 hours of DI the decline of extensors muscles activity was developed. Along with the decline of extensors muscles activity the other features characteristics for supportlessness developed, revealing the consisting order of appearance, namely: a decline of the maximal force-velocity characteristics, alterations in activities of locomotion's control mechanisms.

The results of study allow concluding that the primary response to the withdrawal of support loading is a suppression of activity of the postural muscle system. The other effects of support unloading like the enhancement of flexor mechanisms that is revealed clearly by the increase of flexor muscles electromyographic activity at rest and during maintenance of vertical posture as well as by the increase the amplitude of myographic response of m. tibialis anterior to support stimulation, the decline of force-velocities properties of extensors long with alterations of all related mechanisms, changes of coordination pattern of muscle recruitment appeared to be the secondary phenomena that are implied by the mechanisms of central coupling (like enhancement of flexor activities) or learning (coordination reorganization).

3.1.1.2. Biochemical Markers of Bone Tissue Metabolism in Cosmonauts after a Prolonged Spaceflight

Parameters of calcium homeostasis and its hormonal regulation, including biochemical markers of bone metabolism, were measured in the blood serum of Russian cosmonauts after prolonged flights on the International Space Station during the period from 2000 to 2003. The duration of the spaceflights was 129–196 days. Flight factors had an impact on calcium and bone tissue metabolism after a flight. Increased levels of osteogenesis and resorption markers were detected in the blood of the cosmonauts in the early rehabilitation period after a spaceflight. The prevalence of resorption over the formation of new bone tissue was observed in the early rehabilitation period, when the hormonal system maintaining calcium homeostasis was activated.

Under conditions of weightlessness, specific negative changes in mineral turnover and bone tissue occur in humans. Spaceflight conditions cause a loss of the mineral component of bone tissue and structural changes that decrease the tissue strength. These changes manifest as a negative calcium balance in bone tissue and in the entire body under conditions of weightlessness or earth-based models of microgravity.

There are several biochemical markers for evaluating bone tissue metabolism: bone-specific alkaline phosphatase, osteocalcin (OC), oxyproline, and hydroxypyridinoline cross-links in bone collagen. OC, also called bone Gla protein, is a noncollagenic protein specific for bone tissue and dentin. Its function is not completely understood. OC is synthesized predominantly by osteoblasts and is incorporated into the extracellular matrix of newly formed bone tissue. A portion of this protein permeates into the blood, where it can be measured. Circulating OC has a short lifetime and is rapidly excreted by the kidneys. During puberty, the OC level in human blood correlates with the skeleton growth. OC is elevated in some diseases associated with an increased rate of bone remodeling. OC can be a specific indicator of bone formation because bone tissue is the main source of blood OC.

The mature form of bone collagen has pyridine cross-links, which are liberated during resorption and undergo no further transformations. These post-translational covalent bonds between peptide chains are formed by lysine residues and stabilize the collagen molecule, providing for its especial strength. In connective tissue, the concentration of hydroxypyridinoline cross-links is very low and varies greatly depending on the type of this tissue. Since bone tissue has the highest content of collagen matrix and the metabolic rate is considerably higher than in other types of connective tissue (e.g., in cartilages), it is considered the main source of hydroxypyridinoline cross-links in biological fluids. These cross-links are released from the bone matrix during its destruction by osteoclasts. About 40% of them are excreted with urine in the free form and 60 %, in the peptide-bound form. In osteoporosis, the concentration of hydroxypyridinoline cross-links in the blood correlates with the rate of bone metabolism, which can be assessed by investigation of calcium kinetics or by histomorphometry.

The time course of bone metabolism markers as a function of spaceflight factors was studied for the first time by Russian and US specialists in a collaborative Mir-NASA project. Tests performed during prolonged spaceflights revealed increased renal excretion of calcium and hydroxypyridinoline cross-links and a negative calcium balance in bone tissue.

Further experiments were performed on the International Space Station (ISS). During flights on the ISS, a new integrated system was applied to prevent disturbances in the cardiovascular and motor systems. This system included methods developed by Russian researchers and some new methods. Detection of biochemical markers of bone metabolism in the blood has the advantage of providing more complete data.

This study was designed to check the following ideas: (1) the loss of the mineral component of bone tissue under conditions of microgravity is related to collagen destruction and (2) the recovery of the normal balance between the processes of bone tissue remodeling after a prolonged spaceflight has various rates.

Materials and methods

We investigated blood serum from nine cosmonauts who performed spaceflights on the ISS for 129–196 days. Blood samples were obtained during routine medical examination (a) 30-45 days before the start, (b) the day of landing, and (c) 7–8 and (d) 15–19 days after landing. The blood samples were collected from the antecubital vein into vacuum tubes (S-Monovette) without a preservative. The blood was centrifuged at 2000 *g* for 20 min; the serum was collected and stored frozen (-70°C) in aliquots. Moreover, during the flight, the cosmonauts themselves collected samples of capillary blood for testing with an i-STAT portable biochemical analyzer (United States).

Concentrations of OC and collagen fragments were measured with a commercial reagent kit (Roche) by electrochemiluminescence immunoassay using an Elecsys 2010 apparatus (Roche). This method is based on a two-step sandwich enzyme immunoassay.

The activity of the system regulating calcium turnover in the blood was assessed by the levels of parathyroid hormone (PTH) and calcitonin (CT) measured by routine enzyme immunoassays. Total calcium and its ionized fraction were measured using an EasyLyte Calcium OS electrolyte analyzer for biological fluids (Medica).

The results were analyzed by standard methods of mathematical statistics.

Results

On the day of landing after the flight (day 0), the mean OC concentration was higher than the baseline by 10.7 %. On days 7–8 after landing, the OC level remained elevated (by 27.0 %) as compared to the value before the flight. On days 15–19 after the flight, the mean OC concentration was higher than the baseline by 25.1 %. The mean group concentration of collagen fragments on the day of landing was higher than the baseline by 81.8 %. On days 7–8, the concentration of this marker had decreased, but it did not reach the preflight level, exceeding it by 74.7 %. On days 15–19 of the rehabilitation period, the blood concentration of

cross-links had decreased; however, it exceeded the baseline by 49.5%. These data possibly reflect the maintenance of a high activity of bone tissue resorption (compared to the baseline) despite the cessation of microgravity, the main unfavorable factor.

Thus, the concentration of both markers in the blood of the cosmonauts was higher after landing than before the start under normal vital activity. This result can be explained by the persistent activation of both processes, bone tissue resorption (an increase in the concentration of cross-links) and formation *de novo* (an increase in OC concentration), after a spaceflight. However, the time-dependent changes in the markers after a flight were reciprocal: while the increment in the concentration of collagen fragments decreased during the rehabilitation period, the increment in the OC concentration increased. Hence, the activity of destruction of bone collagen slowly but steadily decreased after the end of a prolonged spaceflight, whereas the activation of remodeling (new bone synthesis) had a latency period and lasted for a long time.

The total concentration of calcium after the flight did not exceed normal values and did not differ from the values before the flight. However, its ionized fraction tended to increase during the flight and the early postflight period. Hence, the increased levels of both mineralotropic hormones in the blood of cosmonauts after a spaceflight reflected functional stress in the homeostasis-maintaining system.

Discussion

In humans, adaptation of calcium turnover to space-flight conditions and readaptation after landing involves all processes of calcium metabolism and is regulated by multiple hormones. Changes in the values of hormonal regulators after prolonged flights revealed that calcium metabolism was affected not only by PTH and CT but also by elevated cortisol concentrations. Facilitated mobilization of calcium from the bone depot (owing to the effect of PTH and cortisol) leads to ionized calcium hypercalcemia, which is constantly observed after a flight. A common manifestation of a negative calcium balance is increased calcium excretion by the kidneys as a result of decreased calcium reabsorption in the renal tubules. A negative calcium balance between the body and its environment is also related to its decreased absorption in the gastrointestinal tract because of the effect of microgravity. This effect was observed in tests performed on the orbital station *Mir* and during the flight of *Skylab*. The level of intestinal calcium absorption was lower and calcium excretion via the intestine was higher even after the end of prolonged spaceflights. After landing, two or three months were not enough for renal and intestinal calcium excretion to reach normal values.

An analysis of the calcium loss by the body in association with a study of hormonal regulation in Russian cosmonauts after flights of 150–237 days on the orbital station *Mir* revealed a relationship between the activity of mineralotropic hormones and the extent of the calcium loss. An increase in the PTH level (sometimes by a factor of 2) seemed to facilitate mobilization of osteoclasts and inhibition of the osteoblast activity in bone tissue. However, the plasma level of

PTH did not correlate with changes in the calcium balance after a one-year spaceflight.

The study of changes in bone metabolism markers during and after a prolonged stay of cosmonauts under conditions of weightlessness has begun recently. Our data show that the recovery of the normal balance between the processes of bone tissue remodeling (i.e., the balance between resorption and bone tissue formation *de novo)* has different rates. Yet, in some cosmonauts, we observed a strong correlation between the levels of collagen fragments and ionized calcium in the blood after a spaceflight (r = 0.86–0.98). This finding means that destruction of the collagen matrix of the bone (i.e., activation of resorption) in these subjects makes the greatest contribution to the deviation of the homeostatic constant of the mineral turnover. In other cosmonauts, this relationship was weaker. Obviously, the loss of the mineral component by bone tissue was related to collagen destruction.

Thus, measurements of markers of bone metabolism in the blood of cosmonauts after a spaceflight, along with investigation of calcium homeostasis and its hormonal regulation by mineralotropic hormones, provide important data on bone remodeling. These data have theoretical and practical implications: a decrease in the mineral saturation of bone tissue and remodeling of its structure under the influence of a spaceflight are adaptive during exposure to microgravity and reversible. On the return of cosmonauts to Earth, these processes begin to reverse, with the initial state observed before the flight eventually being attained. Further tests are necessary to find out the duration of this process and the rate-limiting components.

We are grateful to the Russian cosmonauts who participated in this study before, during, and after flights on the ISS for their goodwill and patience.

The study was supported by the Russian Space Agency and the Russian Academy of Sciences.

3.1.1.3. Results of Microbiological Studies of the Environment of the International Space Station

The development of astronautics in recent decades has been marked by substantial results. One of the chief results in this field has been the creation and long-term functioning of orbital space stations, which are impossible to maintain unless there exists an environment that is optimal in terms of all of the involved variables.

Microorganisms, the automicrobiota in humans, and also the residents of soil, water, and air, all isolated from the biosphere existing within the boundaries of a hermetically-sealed space, are the constant ecological partners of humans during manned space flight. Among these microorganisms, heterotrophs have considerable relative significance. They have the capacity, along with saprophytes, to engage the human body in a diverse interaction – ranging from such forms of symbiosis as mutualism to such phenomena as parasitism and infectious disease. In

addition, as was established during many years of the MIR space station operations, bacteria and fungi would frequently be found within the environment, primarily on the construction materials of the interior and the hardware. As a result of their metabolic processes, they are capable of causing biodeterioration of polymers and biocorrosion of metals. At the same time, the formation of specific reservoirs of microorganism aggregation and reproduction has been noted in specific areas, leading to a number of cases where there was a negative impact on operations and even the failure of various equipment.

In connection with the above, there is obviously an urgent need to study the specific features relating to the formation and behavior of microbiota in manned spacecraft, along with an evaluation of the risks associated with the life processes of microorganisms in the environment. Obtaining such data is a necessary condition for creating a science-based system of ecological monitoring and antimicrobial protection that will fit present and future space missions.

Materials and methods

Over the course of the ISS uninterrupted operations, microbiological testing of the air environment and surfaces of the interior and hardware of the habitable compartments is done on a regular basis.

A study of the aerosol phase of the habitable modules of the ISS was done within the framework of a standard onboard procedure, the "Control of Environmental Microecosphere" (MO-21).

Sampling of airborne microbiota was typically done no less frequently than once every 3 months.

The Russian "Ecosphere" unit includes the SAS air sampling device by PBI International, which is adapted for space flight and uses an aspiration/sedimentation method to collect air samples. It also includes sets of Petri dishes with nutrient media: Medium No. 1 being trypcase-soy agar – for isolating bacteria, and Medium No. 2 being Czapek's medium – for isolating the mold forms of fungi. There is also a Cryogem-03 refrigerator/incubator and an insert with a list of the areas in which to collect samples.

The apparatus sets are located on board the ISS, and the Petri dish sets with nutrient media are delivered to the orbital station by transport cargo vehicles as needed. Research on the airborne of the ISS (incubation of cultures, computation of results) is done by the crew directly on board, with the obtained information being transmitted to Earth by radio communications.

To better understand the specific structure of microorganisms, and also to isolate test cultures of bacteria and fungi in order to study them and form collections of strains for further research, air sampling is also done within 1 – 2 days prior to the transport cargo vehicle undocking from the orbital station. After this, the Petri dishes with cultures of microorganisms are sent to Earth for further study.

In order to perform a microbiological assessment of the surfaces on board the ISS, samples are collected from the station interior and equipment within the framework of a standard onboard procedure, called "Sanitary-Epidemiological

Status Monitoring" (MO-22). Sampling is done within 1 – 2 days before the completion of each mission. In addition to investigations performed within the sphere of medical monitoring, microbiological testing of the Functional Cargo Block (FGB) and Service Module (SM) is also done periodically.

Microbiota sampling of the interior surfaces, instrumentation and equipment is accomplished using the "Test Tube Kits for Microbiological Sampling," which are delivered to the ISS by logistical cargo vehicles before the start, or in the process, of the mission. The Test Tube Kit is a pouch with affixed, fluoroplastic test tubes having pads that are impregnated with a preservative agent, and with an insert listing the areas of sample collection.

Sample collection is done by swabbing off a surface from a 10×10 cm area. Delivery of the Test Tube Kits to Earth, along with the samples collected for further laboratory research, is accomplished with the return of the crew that is being relieved.

In the laboratory, inoculation of the samples collected using the Test Tube Kits is done on the surfaces of nutrient media poured into Petri dishes. A series of elective and differentially diagnostic media are used for each sample.

After the cultures are incubated in an oven (at 37 ºC for 48 hours for bacteria, and at 28 ºC for 5–7 days for fungi), a count is done of the colonies that have developed in the dishes. Material for performing a Gram's stain is collected from the colonies of each morphological type of bacteria. Identification of the bacteria is done using the Vitek-60 automated system by Bio Merieux (France).

After counting the fungal colonies and marking individual species and strains, they are further inoculated onto special media in order to do the identification. For identifying the separate strains of fungi, domestic and foreign fungal identification guidebooks are used. The identification of yeasts and yeast-like fungi is done using the Vitek-60 automated microbiological analyzer by Bio Merieux SA (France).

According to the results of monitoring the microbiota of the ISS environment, the Russian side prepares a summary that is relayed to the members of the environmental habitat working group. If the microorganism content in any particular zone exceeds the standards regulated by the SSP 50260 MORD document, a radiogram is sent to the crew with recommendations for the antimicrobial treatment of the zone in question. For this purpose, a special agent called "Fungistat" is available on board.

Results

In the process of long-term operations of the International Space Station, research has been regularly carried out to study the quantitative content and species composition of the microorganisms forming in the crew compartments. A total of 70 species of microorganisms have been found in the environment of the orbital complex, 36 species of which were bacteria, and 34 species of which were fungi.

For the systematization of bacteria isolated from the ISS environment, we used the classification system contained in the bacteria index, Bergey's Manual of Determinative Bacteriology, Ninth Edition, 1994. The results of identifying the

bacteria found in the ISS environment are shown that all the bacteria isolated from the ISS belong to one of 5 groups: 4, 5, 17, 18, and 20. A total of 14 genera were isolated from the ISS environment, including 36 species of bacteria.

The species composition of the bacteria found in the ISS was distinguished by its considerable diversity. Principally, these were Gram-positive cocci, Gram-negative aerobic or facultative aerobic and spore-forming bacilli. Bacteria of the genera Staphylococcus (12 species) and Bacillus (6 species) stood out as having the greatest species diversity. The largest number of bacteria species was isolated from the interior and equipment surfaces. There were 35 species (97.2 % of the total number of found bacteria species) discovered on the interior surfaces, whereas 15 species (41.6 % of the total number of species) were detected in the air.

Out of 181 samples taken from the environment for the study of the bacterial flora (surfaces, air) of the ISS, bacteria were discovered in 132 samples, amounting to 72.9 % of the total number of samples. Bacteria of the *Staphylococcus* genus were predominant according to the detection rate both in samples that were taken from the surfaces of the interior and equipment (84.0 %), and also in air samples (38.8%). Taking second place in the surface incidence rate were the bacteria of the *Bacillus genus* (31.7 %). *Corynebacterium* came in third (9.4 %), and *Micrococcus* was in fourth place (7.9 %). In all instances, the detection rate for individual genera of bacteria on the interior and equipment surfaces was higher than in the air. The most frequently encountered species in both the air and on surfaces was *Staphylococcus* epidermidis. The incidence of its detection on the surfaces of the interior and equipment was 22.4 %, and in the air it was 9.5 %. On surfaces, the following species were primarily detected: *Staphylococcus auricularis* (23.4 %), *Bacillus sphaericus* (12.1 %) and *Staphylococcus hominis* (9.3 %). Among the bacterial flora isolated from the ISS environment were species identified as belonging to the opportunistic microorganisms capable of causing various disorders when the human body is in a state of immunodeficiency. Mainly, this is *Staphylococcus aureus*, *Streptococcus sp.*, and *Bacillus cereus*, belonging to Group 4 of pathogenic risk. Also encountered among the bacteria isolated from the ISS environment were microorganisms that are known, according to data from the literature, as active causative agents of the biodeterioration of materials of various chemical composition.

For systematization of the species of fungi detected in the ISS environmental habitat, we used the classification system adopted in the Mycological Dictionary (8^{th} edition). The fungal component of the International Space Station contained representatives of 2 taxons of fungi: *Ascomycota* and *Mitosporic fungi*. Imperfect fungi were characterized by the greatest generic diversity among the fungi detected in the ISS environment. A total of 11 genera of mold, yeast like fungi, and yeasts were identified. Considerable species diversity was noted in the *Aspergillus* group (16 species), *Penicillium* group (5 species) and *Cladosporium* group (4 species). The greatest number of fungi species was isolated from the surfaces of the interior and the equipment of the station, 33 species, whereas only 6 species were detected in the air, amounting to 97.0% and 17.6%, respectively, out of the total number of fungi species discovered in the ISS environment. Hence, 34 species of fungi were

found in the ISS environment. These belonged to 2 taxons and 11 genera. Fungi of the *Aspergillus* and *Penicillium* genera were characterized here by the greatest species diversity.

Out of 197 samples taken for studying the fungi of the ISS environment (surfaces, air), fungi were discovered in 89 samples, amounting to 45.2 % of the total number of samples. Fungi of the *Aspergillus* and *Penicillium* genera predominated by detection rate both in the samples taken from the interior and equipment surfaces, as well as in the air samples. As a whole, these values reached, respectively, 19.7–4.9 and 10.2–2.5 % of the total number of collected samples. According to the detection rate on the surfaces of the orbital complex, the following species were predominant: *Aspergillus phoenicis* (6.5 %), *Penicillium aurantiogriseum* (6.0 %) and *Aspergillus sydowi* (3.3%). The most frequently extracted species of fungi from the air were *Aspergillus flavus* (2.5 %) and *Penicillium aurantiogriseum* (1.7 %). Among those fungi isolated from the ISS environment, species of fungi that belong to the opportunistic category were encountered. These are capable in certain conditions, mostly against the background of suppressed immunity, of causing various pathological processes in humans (toxic and allergic disorders, mycoses, etc.).

Out of the 34 species of fungi isolated from the ISS environment, according to the literature data, 10 species belong to the opportunistic variety. This amounts to 29.4 % of the total number of detected species, of which all are pathogens of allergic processes; 7 species of fungi are potential pathogens of mycoses and 4 species are active toxin producers. It should be noted that these fungi are cosmopolitan; they are encountered on all the continents, grow on organic substrates, in the soil and on plants, and their spores are constantly entering the air. However, the mycoses caused by opportunistic fungi develop, as a rule, when there is a serious debilitation of the body's defenses. The elements most often emerging as predisposing and stimulating factors are chronic illness, long-term treatment with broad-spectrum antibiotics, steroid therapy, congenital or acquired immunodeficiency, leukemia, malignant tumors, hormonal and metabolic disorders, etc.

Of the fungi isolated from the ISS environment, considerable relative significance is had by the mold fungi, known according to the data of the literature as active causative agents of the biodeterioration of materials of various chemical composition. Thus, more than 60 % of the fungi found on the interior surfaces of the station are able to participate in the biodegradation processes of polymer materials, and such species as *Penicillium aurantiogriseum* and *Cladosporium herbarum* are, additionally, potential agents of the biocorrosion of metals, as they excrete “aggressive” products of metabolic activity during their growth process. Due to these products, corrosive environments are created on metal surfaces.

Hence, the majority of species of fungi detected on the surfaces of the International Space Station belong to the group of potential causative agents of polymer material biodeterioration, and to the group of metal biocorrosive agents.

Thus, as the result of onboard experiments performed on the International Space Station during the working period of 9 expedition crews and 7 visiting

crews, 70 species of microorganisms were isolated and identified from the environmental habitat. Present among them were both opportunistic bacteria and fungi, and technophiles capable of causing biodeterioration of polymer materials and biocorrosion of metals.

1.1.4. Spaceflight effects on consecutive generations of peas grown onboard the Russian segment of the International Space Station

In 1997–1999, we performed experiments with *Brassica rapa L.* and wheat plants of Apogee var. in the Svet greenhouse onboard Mir. The results indicated that plants could grow in space over consecutive generations. The fact that plants, whose embryological and post-embryological stages developed in space, reached maturity demonstrated that plants could go through many growth cycles in microgravity. However, it was not possible to predict potential changes that may occur over many generations of plants grown in space. In view of this, a study of microevolution and remote genetic consequences of plant growth in space is of high priority. Many authors put forth a hypothesis that prolonged plant exposure to cosmic radiation, man-made atmosphere and other spaceflight effects may cause genetic and micro-evolutionary changes.

In March 2003 – April 2005, we completed 5 experiments using genetically marked dwarf pea plants [9, 10]. The purpose of the experiments was to study morphological and genetic parameters of pea plants grown over several generations in the Lada greenhouse onboard the ISS RS.

The genome of higher plants is of a large size and complex organization. It can be successfully investigated using molecular methods of DNA analysis [11]. Application of molecular methods in biological research provides better capabilities for studying genetic diversity and intra- and interspecies relations. At present, DNA amplification with random or specific markers is widely used allowing detection of variability of many loci across the entire genome.

A commonly applied method of RAPD (Random Amplified Polymorphic DNA) is based on the use of PCR (polymerase chain reaction) with random 9–10 unit primers characterized by predominant G/C (60–80 %) and relatively low annealing temperatures. As a rule, RAPD primers yield 3 to 15 amplification products. DNA segments amplified by RAPD represented as replicate sequences spread across the entire genome.

This paper focuses on the first in the series of pea space experiments and describes plants grown on the ground from seeds that developed in space.

Materials and methods

In the experimental series Plants-2/Lada-2, 3, 4, 5, 6 pea plants were grown in the Lada greenhouse installed on the ISS RS in October 2002. The Lada greenhouse was manufactured for plant experiments to be performed during ISS construction. The greenhouse was small in size (with the crop area of about 340 sq. cm) with low power requirements (about 60 Wt) because of limitations on crew

time, hardware mass and power, which are particularly strict at the ISS assembly stage (Fig. 1).

Fig. 1. Greenhouse Lada onboard Russian Segment of ISS

The experiments were performed on genetically marked dwarf lines of peas (*Pisum sativum*) from the collection of the Genetics and Selection Department of the Biology School of the Moscow State University. Line 102 plants are 15–22 cm high with acacia- like leaves, white petals and yellow pods. Line 131 plants are 25–30 cm high with leaves transformed into branching tendrils, pink-purple petals and green pods. In the root module, 8–10 seeds were planted, which yielded 5-6 mature plants (due to the small crop area). When each experiment was completed, pods containing peas were harvested from some of mature plants and placed in silica gel containing plastic bags. These seeds were later planted in the Lada greenhouse to produce next generation plants. The remaining dry plants with seeds were placed in silica gel containing plastic bags and returned to Earth for further analysis.

With the purpose of identifying the factors that may cause chromosome mutations in plants long exposed to the space environment, chromosome aberrations in rootlets grown from space seeds were examined. Air-dry seeds of the two genetically marked lines were wetted in Petri dishes on filter paper at room temperature. After 48 hours rootlets were fixed in a mixture of ethanol and acetic acid. Fixed rootlets were stained with freshly prepared carmine, crushed and examined by the anaphase method. When examining preparations, cells with single and double fragments, single and double bridges, as well as cells with delayed chromosomes were counted. Seeds of the same lines but grown on the ground served as controls.

Genetic analysis was performed on 5 plants of Line 102 (acacia-like leaves) and 7 plants of Line 131 (tendrils) grown from the first space generation. As controls, 5 plants of Line 102 and 7 plants of Line 131 grown under the same

conditions as the target plants, as well as 2 plants of Line 102 grown in the ground were used. Total DNA was isolated from young leaves using STAV isopropanol method.

For DNA-polimorphism analyses ten 10-unit RAPD primers (AD04, B340, Pr10, B318, R03, OP04, QR2, K10, K8, E16 (Syntol Russia and DNA-Technology Russia) were used. Amplification was performed in a Tercik amplifier (DNA-Technology Russia) as follows: pre-denaturation at 94 °C for 2 min 30 s; 5 cycles at 94 °C for 30 s, at 40 °C for 30 s, at 70.5 °C for 1 min 20 sec; 35 cycles at 93 °C for 20 s, at 37 °C for 30 s, at 71.5 °C for 1 min; final elongation at 72 °C for 5 min. The reaction mixture of 25 μl contained 2 units Taq-polymerase, 10 pM primer, 2.5 mM Mg^{2+}, and 0.25 mM dNTP. The mixture was layered with 2 drops of mineral oil.

Amplification products were separated by gel-electrophoresis (2 % agar gel in 1x TBE buffer) and stained with ethidium bromide. Fragment lengths were measured using markers of molecular mass 100 bp + 1.5 Kb DNA Ladder and pBR322/AluI (Sibenzyme Russia). Gels were analyzed in UV and photographed by a digital camera.

Results

During the ISS Expedition-6 (Fig. 2) an experiment with pea lines 102 and 131 was performed (Fig. 3). The purpose of the experiment was to grow plants over the entire cycle, collect seeds of the first space generation, and then select the pea line, which will be most suitable for future experiments. According to the experimental protocol, one seed of Line 131 and eight seeds of Line 102 were planted. Their germination rate proved to be 100%. The duration of the entire ontogenetic cycle and its stages in space did not differ from that on the ground. As a result, normal plants with pods containing mature seeds developed (one plant of Line 131 and five plants of Line 102). Morphometric examinations of space and control plants did not reveal any differences.

Fig. 2. Flight-engineer ISS-6 Nicolay Budarin conducted the experiment ‘Plants-2/Lada-2’ (20.03.2003)

Fig. 3. Plants of two different lines of pea in flight experiment 'Plants-2/Lada-2'. Left — pea line 131, right — pea line 102 (20.03.2003)

Our examinations have shown that Line 131 plants better suit space experiments because Line 102 plants developed a very thick canopy inside the Lada greenhouse, which significantly reduced their illumination and ventilation. Based on these observations, Line 131 plants were used in all subsequent experiments on ISS RS.

This experiment was followed by 4 other Line 131 experiments (Fig. 4). The total duration of a full plant growth cycle was 73–76 days. The plants were 20-27 cm high, the number of mature peas was 2–4 per plant. The dry mass of a seed (there were 15 seeds altogether, which were returned to Earth after the first, second and third growth cycles) was 0.25±0.06 g. It should be mentioned that crewmembers randomly selected 2 peas at the end of each cycle. The dry mass of a seed from the sample prepared in a similar manner from the control was 0.27±0.07 g.

Fig. 4. Flowers and pods of pea line 131 of the forth space generation (ISS-10, 14.03.2005)

To-datewe have completed a ground control experiment using Line 102 and Line 131 space seeds from the ISS-6 mission. It can be seen that plants grown from space seeds were very similar to those grown from Earth seeds in terms of their growth rate and morphological structure. However, plants from space seeds, particularly Line 102, showed higher production of both vegetative and generative organs, although the difference was insignificant.

Genetic examinations demonstrated high efficiency of the primers selected, some of which identified 9–18 fragments with a length of 210 to 2500 bp. Altogether 124 fragments were obtained, with Lines 102 and 131 differing with respect to 46 fragments (37.1 %). In Line 102 111 fragments and in Line 131– 91 fragments were amplified. Analysis of individual plants showed that Line 131 was uniform in regard to all markers examined, whereas Line 102 was characterized by intra-linear polymorphism with respect to 17 markers (13.7 %). These markers were seen both in space and control plants and were therefore excluded from further variability examinations.

Spectral analysis of space and control plants with respect to markers revealed no visible differences. Each space plant amplified all fragments expected and showed no new fragments. Line 131, in contrast to line 102, was uniform with respect to RAPD markers (Fig. 5).

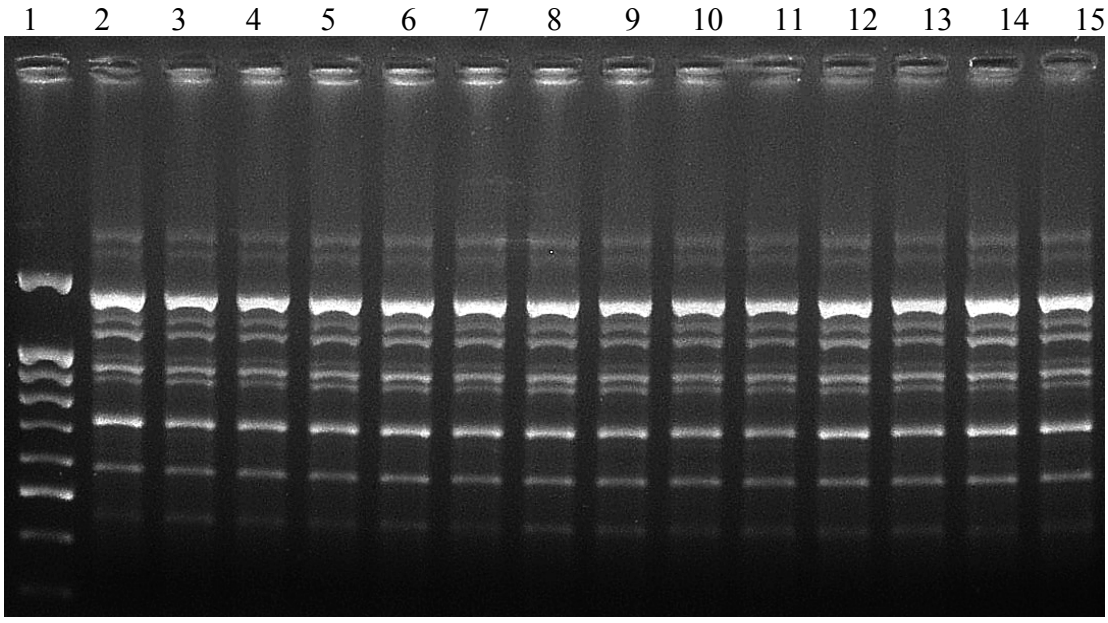

Fig. 5. RAPD spectra of Line 102 resulting from amplification with QR primer. Arrow indicates the marker which showed polymorphism in Line 102: 1 — marker of molecular mass 100 bp + 1.5 kB DNA Ladder; 2–6 and 12–13 — control plants; 7–11 — space plants

Cytological analysis of rootlets of plants grown from the space seeds of the first generation did not show a significant increase in the number of chromosome aberrations in the space-flown plants compared to the controls. It was found that Line 102 rootlets developed a larger number of chromosome aberrations in both space-flown and control plants. Line 102 space seeds showed a 2 % increase of

chromosome aberrations. It can be concluded that the genetic findings gave additional support to our use of Line 131 in space experiments onboard the ISS RS.

Conclusion

Our observations demonstrated that pea plants grown over a complete ontogenetic cycle in space were similar to the ground controls in terms of their developmental and genetic characteristics. Preliminary analysis of Line 131 pea plants grown for four consecutive generations gives evidence that plants can grow for a long time in the space environment and maintain their capability to yield viable seeds.

To-date, genetic analysis of pea plants grown from space and Earth seeds of the first generation is complete. It was performed using the method of RAPD (Random Amplified Polymorphic DNA) with respect to 10 markers, as well as an examination of chromosome aberrations. No signs of genetic polymorphism were detected. This suggests that exposure to the space environment did not cause any genetic changes in pea plants of the first space generation.

3.1.1.5. Effects of space station conditions (ISS) on resting egg and life cycle in D. magna and S. torvicornis

Dormancy is widespread adaptation protected many species of animals and plants in harsh environmental conditions. In aquatic animals this adaptation is especially well developed that provide water living organism with strong unspecific to negative factor defense lasting from months up to hundred years. This adaptation of aquatic organism can be perspective for long-term transportation of ecosystem elements in space missions when renewable source of food and an efficient method to recycle oxygen are required. It was shown that aquatic micro algae are much more effective in oxygen recycling than terrestrial plants and micro-crustaceans can be very value protein source in distant space mission. However, long duration plant growth and reproduction in space that is necessary for transportation of a. control ecological life support system (CELSS) from Earth to other planets are problematic. The introduction of heterotrophs in space CELSS is a more formidable problem as the absence of gravity creates additional difficulties for their life. Dormancy phenomenon protected a great many animals and plants in harsh environmental conditions can be quite perspective as a tool to overcome difficulties with CELSS transportation in space missions.

Effects of space station conditions such as space radiation, strong magnetic/electric fields and microgravity on resting stages of animals and plants have not been studies yet. This study was conducted in order to elaborate a new technology for creating of artificial ecosystem outside the Earth biosphere. Maintaining of aquatic animal and plant resting stages in space conditions will be essential part of this biotechnology.

We examined reactivation of resting stage and life cycle parameters in two perspective for space mission crustaceans species (*D. magna/* and

/S. torvicornis)after a month exposition of their dry resting eggs in the Russian segment of International Space Station (ISS). Special attention was paid to possible negative changes caused by the set of factors at ISS including low gravity, radiation, magnetic/electric field and biological impact of bacterial-fungal flora as well.

Material and methods

Resting eggs of *D. magna* and *S. torvicornis* were collected in artificial ponds for *Daphnia* cultivation used in sturgeon aquaculture in Trudfront State Sturgeon Farm (Astrakhan district, South of European Russia, 46° 00' N; 47° 30' E). The betony ponds exist more than 30 years and regularly operate in May-June during short period of plankton feeding in sturgeon larvae. In the end of June Dapnia's ephippia collected from ponds are placed in fiber bags and kept dry at outside temperature until next spring. During winter they passed through natural cold termination and before use in our experiments these eggs were almost activated and ready for development. Preliminary test in May 2005 indicated high level of reactivation (up to 80 % in *D. magna*) in resting eggs of both species.

In laboratory resting eggs were separately fixed in triplicate on sticky plastic film mounted as special window in carton blocks, cowered with protective paper sheet and placed in zipping plastic envelops to avoid crushing and unsanctioned movement during transportation to ISS and staying in microgravity. Each carton block contained 3 windows with resting eggs of *Daphnia* and the same for *S. torvicornis*. The carton blocks than were randomly spited in 4 groups 9 replicates each: 1. exposed at ISS within one month; 2. exposed at ISS within 8 months; 3. laboratory control (20 °C), natural light; 4. cold control (4 °C, darkness).

At Russian segment of ISS the envelops of 1 and 2 groups laid inside cit Rasteniya-2 with controlled temperature and radiation.

The samples from orbit in 10 days after delivering to the Earth surface in parallel with control ones were transported to Laboratory of ecological physiology in MPG Institute for detailed analyses of their reactivation and productive potential in these two species. Resting eggs were calculated at each plastic film and than placed into Petri dishes, rinsed with cold (10 °C) water preliminary aerated within one hour. Resting eggs than spent two days in dark room at 10 °C for slow swelling.

After this preliminary preparation resting eggs on plastic film were transported into thermostat with fluctuated temperature and day length. During day (14 hours) they stayed at 25 °C and in night time (10 hours) they stayed at 15 °C.

This imitated natural light/temperature conditions in spring. The dishes were checked twice per day and all reactivated animals were picked up, pictured under microscope with a digital camera and than used for growing experiments or dried up (60 °C, 24 h) and weighted at "Sartorius" balances with resolution 0.0001 mg. Usually not single neonate but small groups 5–10 individuals each were used for weighting. The pictures than were used for neonates length getting on PC with Adobe measurement tool with resolution 0.001 mm.

Testing of reactivation lasted at least 3 days after the last neonate appeared (normally 2 weeks). Resting eggs in the same dishes were than returned to cold (10 °C) and dark room for 2 weeks. After the cold treatment the same cycle of reactivation were repeated again. We calculated rate of reactivation for every day and total amount of hatched neonates from number of resting eggs.

Oospores of daphnia's parasite fungi *Pitium daphniarum* were applayed to test level of resistance in *D. magna* embryos. Oospores were obtained from a wild culture of *Pitium daphniarum* via cultivation of its mycelium in drying drop of water in cold temperature within 3 days. Presence of oospores in the culture were checked under microscope. Than mycelium were cut in equal portions that were added to Petri dishes with 10 *D. magna* embryos at the first stage of cleavages. We tested resistance of embryos to parasite invasion both in exposed and control groups in 4–6 replicates each. As a reference outside group we also used *D. magna* embryos with suppressed reactivation rate. This reference group were kept in bad conditions (high temperature, constant light, dry air) within 6 month.

Daphniids and phyllopods were cultivated in groups of 4-6 individuals in 250 ml flow-through vessels (Lampert et al., 1988) at a flow rate of 1 l day)1 under constant food and photoperiod conditions at 25 °C. Crustaceans were fed the green alga Scenedesmus obliquus (strain SAG 276-3a, algal collection, Göttigen) grown in a chemostat with modified CHU-12 medium (Müller, 1972) at a constant rate of 0.7 day)1 to maintain equal quality of food for all treatments.

An experiment started with placing neonates in high 1 mg carbon l-1 food concentration with long (14 h) and short (10 h) day photoperiod.

The food medium was replaced every morning and the animals were checked twice per day. After the females had released their first clutch, we recorded the time, counted the neonates (size of first clutch) and determined the neonate dry mass. Females were cultivated under these conditions until they deposited their second clutch of eggs in the brood pouch. Then they were also dried and weighed. Neonate mass and final female mass were used to calculate the somatic growth. Several females from control and ISS-exposed were used to obtain the third clutch which neonates were than cultivated at the same conditions their mother laid eggs. After maturation they were checked on sex, size and dry mass.

The data obtained were not always normally distributed, hence, after a test of the assumptions we used either a t-test or a nonparametric test (Mann–Whitney U-test, Kruskal–Wallis test) for the assessment of significant differences. Statistics were run using Statistica-6 program.

Results and Discussion

Hatching of juveniles from ISS treated ephippia and in control started on third day after moving from 10 to 25 °C (fifth day after beginning of activation) and on fifth day (7^{th} day after start of activation) from embryos kept in bad conditions. Next day in first two variants the maximum of the process in ISS treated and in control was observed and within 7–11 days (longer in control) the hatching rate gradually declined to zero. In former variant the maximal of hatching happened close to the expiring of the process that stopped abruptly at sixth day

after beginning. The average reactivation rate in ISS treated embryos was 39.6 % and in control significantly higher 51.8 % ($p = 0.035$) if calculate to the ephippia number. As among ephippia we found a variety in embryo number (about 13 eggs per 10 ephippia) the real reactivation rates were 30.6 and 39.9 % correspondingly. In embryos kept in bad conditions the real reactivation rate was significantly less than in previous variants (19.0 %).

Beside the differences in reactivation rate and duration of this process the tested groups also are divided in dynamics of neonate dry weights. In control group neonate dry weights in the beginning of hatching period (1–3 days) the neonates were significantly smaller than in the middle (4–5 days) of the process (*t-test* $p = 0.02$). In ISS treated embryos we found a vice versa neonate dry weight dynamics, the difference also was statistically confirmed by t-test ($p = 0.0174$). Actually in control group neonates with small dry weight (0.0049 + 0.00047 mg) hatched first when in ISS treated group the biggest embryos (0.0056 + 0.00034 mg) appeared first. This difference was statistically confirmed by t-test (p=0.0201). In the middle of the hatching period in control we found bigger neonates (0.0056 + 0.000418) than in ISS treated group (0.0046 + 0.000579), this difference was also confirmed by *t-test* ($p = 0.002$).

At the same time the average dry weights in these two groups calculated for all period of hatching in spite a relatively big size of sample (34 weights for two groups) was not significant ($p = 0.504$).

We proposed that at ISS *D. magna* embryos was treated by a negative factor or by set of factors that suppressed their vital ability so the most weak embryos with lower weight could not hatched and their place in ISS group was taken by large size neonates possibly also with suppressed vital ability.

Hatching of phyllopod from ISS treated cyst and in control started simultaneously next day after moving to 25 °C. The clear seen difference in total duration on hatching (ISS 3 days, control 9 days) between these variants was also accompanied by significant difference in total reactivation rate. As in Daphnia case reactivation ISS treated cysts was about twice less than the same in control (t-test $p = 0.034$). Cysts kept in bad conditions had reactivation efficiency close to zero. Neonate dry weight was only traced in control group. The biggest neonates hatched at the first day than an average weight of neonates declined gradually.

To check our hypothesis on lower vital ability in Daphnia embryos exposed at ISS than in those kept in control we tested resistance in several groups of eggs to a fungal parasite's invasion (*Pitium daphniarum*). Two parameters were used: 1. intensity of infection, calculated as number of eggs infected by parasite, %; 2. widespread of infection, calculated as number of variants successively infected by parasite, %. Two groups of embryos exposed at orbit were more sensitive to parasite invasion than those from control. All these three clear differed from embryos with indeed suppressed reactivation rate cause by inappropriate storage conditions especially in sense of widespread of infection. In intensity of infection ISS exposed samples were more close to embryos with suppressed reactivation rate. The difference in intensity of infection between ISS exposed embryos and control were statistically significant (*t-test* $p < 0.04$).

Many aquatic fungi are better known as saprophyte more than real parasite. We used % of reactivation for each group (obtained separately), as a reference for egg capacity. In fact this also could be explained by different rate of egg mortality in these groups as fungi could infect embryos that had died before the test. Hopefully in our experiments we obtained an evidence that the fungi had infected alive embryos that than hatched and lived and even moved within several hours. Also periodically we found new born juveniles surrounded with fungi-mycelium. Such mycelium as we had observed grew 2-3 days, that could mean they also were infected being embryos. This lets us to conclude that *Pitium daphniarum* as a real parasite infected more intensively weaker than in control but alive ISS-exposed eggs.

The experiments were designed to determine effects of resting egg treatment by space factors on life-history parameters in *Daphnia magna* such as time to release of the first clutch, survival to maturation, size of the first clutch, mass of first-clutch neonates, female dry mass at the age of second clutch releasing and possible maternal influence on sex in offspring. We found significant effects ISS exposing for two of these parameters. The first clutch size in females arisen from embryos exposed at ISS (11.14 + 3.592 egg ind^{-1}) was lower than in control group (14.39 + 2.847 egg ind^{-1}) that confirmed by t-test ($p = 0.0477$). Small but also significant difference was found for *maturation time* that was slightly longer in ISS exposed animals, 9.95 + 0.284 day than in those kept in laboratory 10.31 + 0.372, confirmed by Mann-Whitney test (*zadjust* = 2.09, $p = 0.033$). Both parameters are very important in Daphnia population dynamics.They are mainly responsible for fitness of population or clone to environmental conditions and also play key role in their productivity both in culture and in nature.

In spite our expectation there were not confirmed differences in survival to maturation, neonate and female masses as well.

S. torvicornis grown from eggs collected in the pond is characterized very high level of variability. Practically in each replicate one or two definitely were found that affected of course life cycle parameters of the individuals grown in our experiments. Due to this a great variation in *S. torvicornis* grown from the eggs collected in the wild population we could not find any significant differences between experiments done on exposed in space and kept in laboratory diapausing cysts of this species. The only significant effect found in analyses of *S. torvicornis* life cycle was influence of day length on length of animals in age of 12 days (time of appearance of eggs in female brood sac). In long day average length of animals (9.838+1.1546) differed from the same in short day (8.676 + 1.9123) (Mann–Whitney-*test* $z = 2.021$; $p = 0.043$). Other parameters like female/male length or clutch size also showed differences between experiments in long/short day conditions and ISS/control environments but huge population polymorphism resulted in high level of standard deviation and masked these effects to our mind.

S. torvicornis is an obligatory bisexual phyllopod so it is impossible to obtain a genetically homogenous strain like in some Daphnia with obligatory parthenogenesis to decline genetic variation. To overcome these obstacles with population polymorphism in *S. torvicornis* resting eggs it is necessary to get eggs

of the species in controlled environmental conditions including food, temperature and day length.

We traced growing of offspring (third clutch) of females exposed in space and kept in laboratory conditions as diapausing embryos. A remarkable difference in sexual structure of these offspring.between ISS and laboratory kept variants was found. All five replicates of offspring obtained from females exposed in space contained high proportion of males ranged from 30 to 78 % (54.9 + 25.94 %). No a single male had been appeared in offspring from control group.

It is known that shift from parthenogenesis to bisexual reproduction especially in *Daphnia* is a response on environmental stress of different origin like food declining, changes in photoperiod, signal metabolites etc accepted mainly in maternal environment. In our experiments cultural conditions were equal, stable and favor for parthenogenesis in both experimental groups within two following generation. The only clear seen difference between them was in environmental history of diapausing embryos within one month. It seems like the month exposing at space conditions was accepted by the diapausing embryos as a stress and this information was than transmitted via maternal effect to their offspring as it was shown for photoperiod and other biological information in many invertebrates including *Daphnia*.

This gives us more proof to claim on environmental stress obtained in space as also a reason of other differences between ISS treated and control groups obtained in previous experiments on reactivation, parasite resistance and life cycle analyses in *D. magna*.

Sensitiveness of resting eggs to space flight factor has not specified. This can be a result of space radiation, magnetic field or even microgravity. Also these factors can work together and produce a synergetic effect. In such case these negative effects of space exposition for biological material can be very difficult to avoid. This gives additional problems in creation, exploitation and even for transportation of ecological artificial ecosystems outside the Earth biosphere.

Conclusion

In *D. magna* and *S. torvicornis* we found statistically confirmed differences in reactivation efficiency between resting eggs exposed at ISS and control group kept in laboratory.

Embryos of *D. magna* exposed at orbit were more sensitive to fungal infection (*Pitium daphniarum*) than reference group. In culturing experiments *D. magna* juvenals obtained from ISS exposed eggs had longer maturation time and lower offspring productivity than control group. Exposing of resting eggs in space conditions also induced male production in *D. magna* offspring.

S. torvicornis in fact also demonstrated some difference between ISS exposed and control groups of resting eggs during cultivation but most of the differences were not statistically significant due to very high level of population variability in quality of juveniles. The only significant effect found in analyses of *S. torvicornis* life cycle was influence of day length on length of animals in the age of egg appearance of in female brood sac.

3.1.2. GELIOBIOLOGY

1.2.1. Rhythms of helio-geomagnetic activity as an external synchronizers

New results obtained confirming a hypothesis that rhythms of helio-geomagnetic activity were an external synchronizers that formed an endogenous time-structure of biological systems: the rhythms with periods from 0.5 to 2.2 years (quasi-biennial) which are characteristic for Wolfe numbers, magnetic fields of the Sun, solar wind speed and geomagnetic activity indexes Ap and Kp, were discovered in medico-biological data such as an arterial blood pressure, heart rate in humans, and sudden death in various regions of the world.

A spectral-time analysis of a long time series of medico-biological data showed that there are rhythms with periods about 0.5 y, about 1 year, 1.3 y, 1.8 y and 2.2 years in sudden death numbers in various regions of the world, in arterial blood pressure, heart rate in humans as well. These periods are not only similar to aforementioned long periodic rhythms of the solar activity but have a rather typical similar dynamics in the solar cycles approximately following the dynamics of the solar activity sometimes with some delay from the solar activity rhythm bifurcation events (With Halberg F., Cornelissen G., Cantinas G. (USA) and Y.Watanabe (Japan).

3.1.2.2. New scenario for the prebiologic stage of evolution and the universal mechanism of its realization

1. A new universal nature mechanism of abiogenous synthesis of organic compounds (OC) in plasma torch generated in the processes of SHVI is proposed. It is capable to provide generation of complex OC required for origin of life on early Earth during heavy meteor bombardment and synthesis of OC in the interstellar clouds at the SHV collisions of the dust particles.

2. An original scenario of prebiological stage of evolution on early Earth based on new mechanism is proposed. For its realization was used energy of impact and meteorites substance. Solar radiation participation in the processes of synthesis and presence of certain composition of atmosphere was not required.

3. Based on results of experiments on study of plasma torch structure, a hypothesis on possibility of asymmetrical origin of isomers in local unipolar asymmetrical electrical and magnetic fields or under the influence of circularly-polarized radiation, generated in the process of fly away of hot, highly anisotropic and dense plasma is proposed.

4. It is shown that initial conditions in the single act of meteor impact as well as variety of synthesized OC can provide formation of protobiont-type structure, which will be able with a time (in ~300-400 million years), when genetic code and reproduction mechanism appear, to form a living cell.

5. A simple and reliable mechanism that can provide saving, accumulation and transportation of synthesized OC at the significant distances by dense dust clouds and dust deposits formed due to meteor impact crater formation is proposed.