BIOLOGICAL STUDIES IN SPACE

(Organization of preparing and conducting experiments "Plasmid", "Receptor", "Regeneration", "Gecko" in space flight on board spacecraft "Foton-M" No. 2 and in the synchronous earth control)

PARTISIPANTS:

SSC RF Institute of Biomedical Problems, Russian Academy of Sciences (SSC RF IMBP RAS) Moscow

Institute of Genetics and Selection of Industrial Microorganisms (GosNIIgenetika) Moscow

Institute of Higher Nervous Activity and Neurophysiology (HNA and NP), Russian Academy of Sciences, Moscow

Kol'tsov Institute of Developmental Biology, Russian Academy of Sciences (IDB RAS), Moscow

Institute of Human Morphology, Russian Academy of Medical Sciences (IHM RAMS) Moscow

University of Montana, United States

Ames Research Center NASA, United States

```
TsSKB-Progress (Samara)
```

EXPERIMENT "PLASMID"

AIM:

Verification of the probability and extent of the influence of space flight factors (SFF) on the inheritance and structural stability of plasmid pJ702 in actinomycete *Streptomyces lividans*

TASKS:

- to examine the effect of SFF on genetic structures of the microorganism

- to determine the nature and possible mechanism responsible for changes in genetic structures
- to compare stability and expression of plasmid and chromosomal genes under conditions of SFF effect

The object of this study - actinomycete Streptomyces lividans The device "PLASMID " (board container BB-1M)

PARTISIPANTS:

State Scientific Center, Institute of Biomedical Problems, Russian Academy of Sciences, Moscow (SSC RF IMBP RAS) GosNIIgenetika Russia University of Montana, United States

EXPERIMENT "RECEPTOR"

AIM:

Assessment of the degree of alterations in elements of nervous system responsible for susceptibility and realization of the gravitational stimulus

TASKS:

- elucidating the regulatory role of prepro HP gene expression in the process of organism adaptation to conditions of space flight (microgravitation) and readaptation to the gravity on Earth.
- study of changes occurring in gravireceptor cells (statocytes) during space flight
- evaluation of potential changes in the nature of interaction between photo- and gravirecepto

The object of this study - garden snail *Helix lucorum* The device: "RECEPTOR" (board container BB-1M)

PARTISIPANTS:

SSC RF Institute of Biomedical Problems, Russian Academy of Sciences (SSC RF IMBP RAS) Moscow Institute of Higher Nervous Activity and Neurophysiology (HNA and NP), Russian Academy of Sciences Ames Research Center NASA, United States

EXPERIMENT "REGENERATION"

AIM:

Determination of molecular-biological mechanisms responsible for the stimulatory effect of space flight factors (SFF) on cell proliferation and regeneration of organs and tissues in amphibia

TASKS:

to elucidate the role of genes encoding a number of transcriptional factors in the processes of cell proliferation and regeneration of organs and tissues in lower vertebrata - amphibia
to study the blood cellular composition and conduct histological analysis of blood-forming organs
The object of this study - triton *Pleurodeles waltlii*The device "REGENERATION" (board container "Triton")

PARTISIPANTS:

SSC RF Institute of Biomedical Problems, Russian Academy of Sciences (SSC RF IMBP RAS) Moscow Koltsov Institute of Developmental Biology, Russian Academy of Sciences (IDB RAS) Ames Research Center NASA, United States

EXPERIMENT "GECKO"

AIM:

Study of the influence of space flight factors (microgravitation) on morpho-functional characteristics of the nervous system, skeleton, and endocrine organs in reptile

TASKS:

to study changes in the central nervous system and peripheral organs of sense in reptile exposed to space flight (SF) conditions
to examine the elements constituting the bone muscular system in reptile exposed to space flight conditions
The device: "SNAIL" (board container BB-1M)
The object of this study - geckos Pahcydactylus bibroni

PARTISIPANTS:

State Scientific Center, Institute of Biomedical Problems, Russian Academy of Sciences (SSC RF IMBP RAS) Institute of Human Morphology, Russian Academy of Medical Sciences (IHM RAMS) Ames Research Center NASA, United States

BIOTECHNICAL TRIALS OF DEVICE "PLASMID"

In the framework of the program on testing board devices with biomaterial selected on March 2005, biotechnical trials of the board device "PLASMID" (board container BB-1M) were conducted.

Sixteen plastic Petri dishes 60 mm in diameter containing Streptomyces lividans 66 (pJ702) on a solid agar medium were placed in the metallic container. The inner surface of the container and the space between dishes were covered with a thin layer of porolon.

The container with biomaterial was placed in refrigerator at 4 C and kept for 4 days, then, it was placed in the thermostat for 16 days at 25 C. Thus, space flight conditions can be mimicked. Moreover, considering the possible range of changes in environmental parameters within the inner space of the landing module of spacecraft "Foton-M", scientific equipment was constructed within the temperature range of 20 to 30 C and humidity from 40 to 70% The results obtained confirmed the possibility that experiment "Plasmid" can be successfully conducted in flight of "Foton-M" No.2.

BIOTECHNICAL TRIALS OF DEVICE "RECEPTOR^w

To conduct biotechnical trials of the board device "Receptor", a plexiglass inner box was prepared, which had dimensions corresponding to the inner volume of the board container BB-1M with biomaterial (35 snails *Helix lucorum*). To ensure air exchange between the environment (inner volume of the spacecraft) and the box with snails, approximately 20 holes 5 mm in diameter were cut out in side walls of the container and box. To avoid the release into the environment of snail decomposition products in case of their death under space flight conditions, the container was supplied with filters for air purification from harmful contaminants. For the maintenance of a relatively high humidity level and to avoid the presence of free water, a plate made of polivinilformal, which possesses high water-retaining properties, was attached to the support of the container.

In the framework of the program on testing the scientific device "Receptor", biotechnical trials were conducted in April 2005. Animals (35 snails) were kept for 20 days within the board container BB-1M without food and water under conditions of high humidity (90-95%) at 20 to 30 C.

The results obtained in the course of biotechnical trials allowed the conclusion about the possibility of successful conducting experiment "Receptor" in flight of "Foton-M" No.2.

BIOTECHNICAL TRIALS OF DEVICE "SNAIL"

To conduct flight and control experiments "Gecko", the device "SNAIL" (board container BB-1M) was used.

Biomaterial (5 geckos *Pahcydactylus bibroni*) was placed in a special box made of biologically neutral material, which accurately corresponds to the inner volume of board container BB-1M. Holes (5 mm in diameter) were cut in the box and in the container to ensure air exchange between the environment (inner volume of the spacecraft) and the inner volume of the container.

In the frame work of the program on developing methods of pre- and after flight studies in the course of biotechnical trials of the device "SNAIL", 3 laboratory experiments were conducted in January-March 2005. Each experiment lasted for 20 days and was aimed at a determination of the conditions for keeping animals in the limited space of the board container, of their optimal age and weight characteristics, estimation of the number of animals, and designed to assess the necessity of ensuring food and water during space flight. At the completion of trials, the animals were alive and highly active in all 3 experiments. Thus, our results provided convincing evidence proving that lizards (geckos) can live without food and water for 20 days and retain active life and normal physiological parameters under these conditions. These results allowed the conclusion about successful conducting experiment "Snail" in flight of spacecraft "Foton-M" No. 2.

BIOTECHNICAL TRIALS OF DEVICE "REGENERATION«

To conduct experiments (flight and control), the container "Triton" was used. Animals (20 tritons *Pleurodeles waltlii*, each of 10-12 g) were placed in the container. The bottom of the container was covered with a plate made of polyvinylformal and 500 ml water was added. Thus, 100% humidity was achieved in the container. The container lid made of transparent plastic material had a semipermeable membrane consisting of two plates of porous material with the total area of 200 cm2 to guarantee the air exchange between the inner volume of the spacecraft and inner volume of the container.

In the framework of the program on testing the scientific device "REGENERATION", biotechnical trials were conducted at the end of 2004 and in early 2005. In the course of biotechnical trials, methods of pre- and post flight studies were proposed and optimal parameters of the animal habitat under conditions of space flight were established. In addition, as a result of these trials, optimal quantitative, age and weight characteristics of animals were determined as well as the time periods of performance of preflight surgical operations on amputation of extremities and elimination of crystalline lens in some experimental individuals.

The results obtained allowed the conclusion about the readiness of the board device "Triton" to participate in pilot tests and confirmed the possibility that experiment "Regeneration" can be successfully conducted in flight of "Foton-M" No. 2 spacecraft.

PROTOCOL OF CONDUCTING EXPERIMENTS AT TAKE OFF GROUND OF SC "FOTON- M" No. 2.

May 25 (5.30 to 8.30 pm) filling up scientific devices (board containers) "Regeneration", "Gecko", "Receptor"*

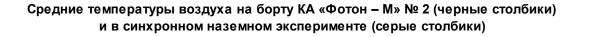
The board device "Plasmid" was filled up May 26, 6 am. Flight containers "Gecko" and "Regeneration" were kept overnight (May 25-26) at room temperature 23-25 C. The container "Receptor" was kept in refrigerator at 4 C. May 26 - transportation of the devices from Moscow to the take-off ground (cosmodrom Baikonur) Kazakhstan May 27 (8.30 pm) - all flight containers are delivered to the assembling-experimental complex (AEC) of SC "Foton-M" No. 2. The temperature within the complex was 20-22 C, humidity 60%.

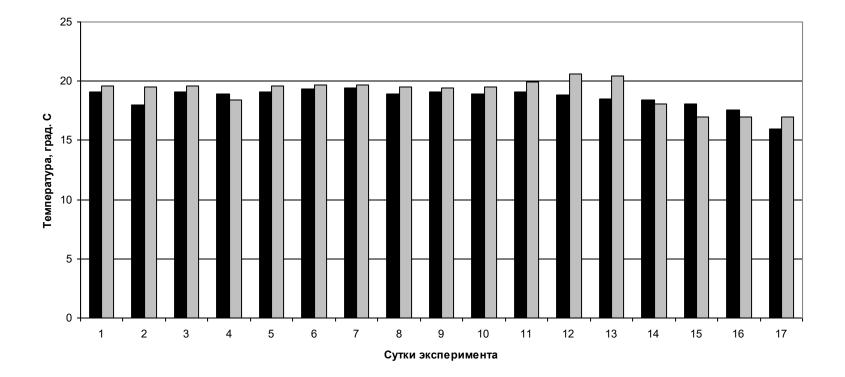
May 27 (10 to 11.20 am) the control of the board devices was provided and the equipment was approved and allowed to be installed in SC "Foton-M" No. 2.

May 28 - 1 to 4 am, assembling of the scientific equipment "Plasmid", "Receptor" and "Regeneration" in SC. * The device "Gecko" is installed in SC "Foton-M" No. 2 on May 27 at 4.15 pm in the upper SC module. (The devices "Plasmid" and "Receptor" are installed in the middle part directly next to minor hatch No. 2, the "Regeneration" device was installed near the large hatch No. 1). May 28 - 9.10 pm, closing the hatches of SC.

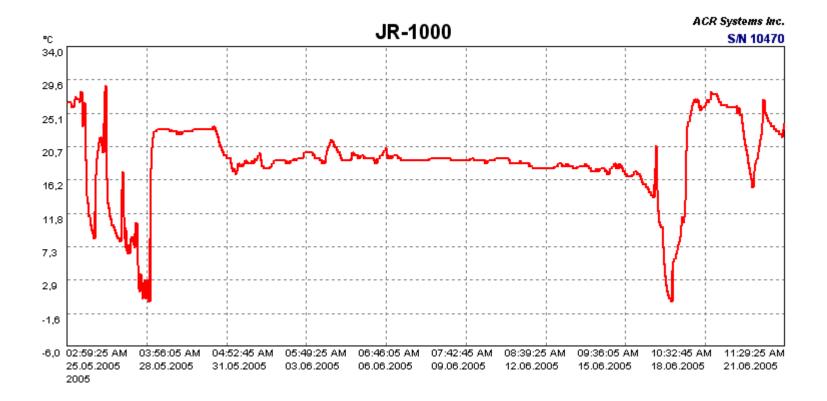
May 29 - junction of SC with the rocket.

May 30 - removal of the SC complex to the take-off ground. May 31 4.00 pm. Take off of SC "Foton-M" No. 2.

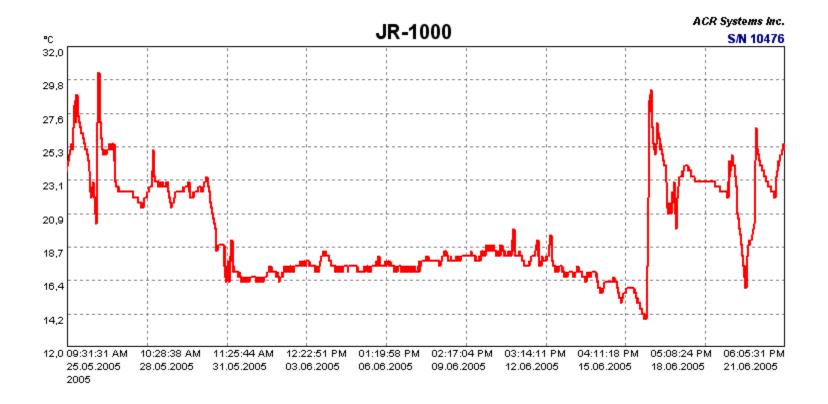




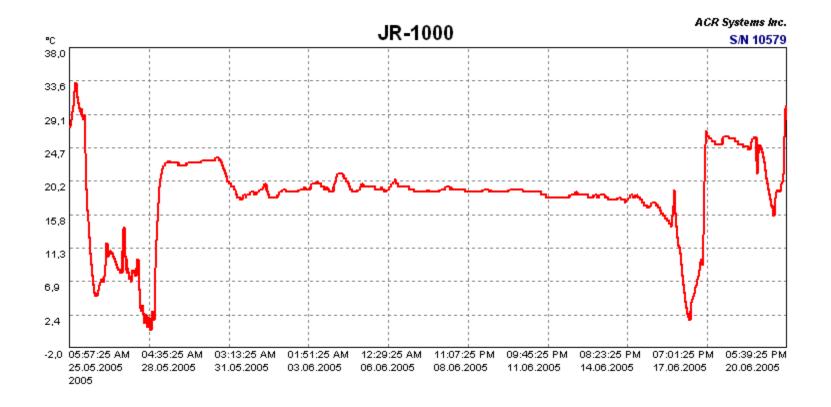
Эксперимент "Рецептор"



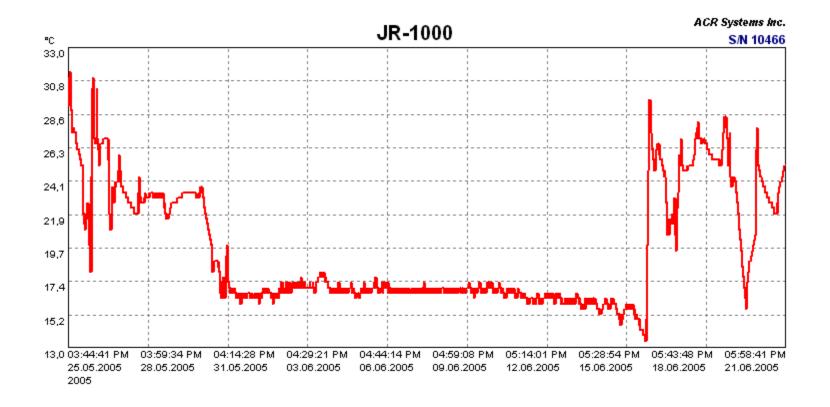
Эксперимент «Регенерация»



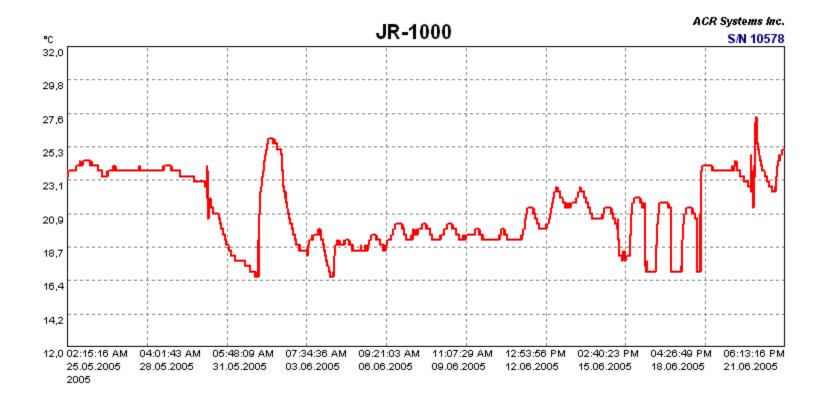
Эксперимент «Плазимида»



Эксперимент «Улитка»



Синхронный «наземный» эксперимент



PROTOCOL OF CONDUCTING EXPERIMENTS AT LANDING GROUND OF SC "FOTON-M" No. 2.

June 16, 11.40 am. Landing of SC "Foton-M" No. 2 in the southeastern part of Kustanai, Kazakhstan. 12.30 opening the hatch No. 1, delivery of container "Triton" (experiment "Regeneration" *All 20 tritons are alive. Transfer of animals to fresh water and substitution of polyvinylformal.

1.15 pm opening the hatch No. 2, delivery of devices "Plasmid" and "Receptor" and their arrangement in the transport thermostat-refrigerator at 4 C.

1.40 pm delivery of device "Snail".

*Devices "Snail" and "Regeneration" are installed in nonthermostating transport container (Air temperature at landing ground was29 C).

June 17, from 7.30 to 11.15 am - flight from the Kustanai airport to the airport of Samara, the flight from Samara to the airport Domodedovo of Moscow - from 12.45 to 3.15 pm.

4.00 pm - the device "Receptor" was passed to A. Malyshev (the scientific worker of HNA RAS) at the airport Domodedovo.

4.55 pm - the device "Plasmid" was passed to V. Tabakov (the scientific worker of GosNIIgenetika).

5.20 pm - the device "Regeneration" was passed to scientific workers of IDB RAS

5.45 pm - the device "Snail" was passed to scientific workers of IHM RAMS

All flight experiments were sent to Moscow and delivered to scientific laboratories 28 h after landing of SC "Foton-M" No. 2.





ORGANIZATION AND CONDUCTING THE SYNCHRONOUS (CONTROL) EXPERIMENT

Synchronous (control) experiments "Plasmid", "Receptor" "Regeneration", "Gecko" were organized and conducted in the laboratory of gravitational biology of SSC RF Institute of Biomedical Problems, Russian Academy of Sciences (SSC RF IMBP RAS) Moscow (the base Planernaya) with the shift of time for 2 days after take-off of SC "Foton-M" No. 2. Thus, control laboratory experiments were conducted from 4 pm of June 2 till 11.30 am of June 18. Note that the time shift for 2 days after the start of flight experiments was caused by two factors: first, it was necessary to obtain information about environmental parameters, primarily, the temperature in the inner space of SC "Foton-M" No. 2, and second, for ensuring the time required for processing and express-analysis of afterflight material following its delivery to Moscow after landing of module of spacecraft "Foton-M" No. 2.

All 4 containers with biomaterial identical to board (flight) containers (after procedures analogous to preflight procedures) were placed into the special laboratory thermostat (climatic chamber) of the company Heraeus, where they were kept for 16 days. At definite time intervals (2 h), the needed corrections of temperature and humidity parameters were made after information from the Center of flight governing. All containers in the control experiment, like flight containers, were kept in dark. Changes in temperature and other parameters of flight and synchronous experiments are presented in the corresponding charts.