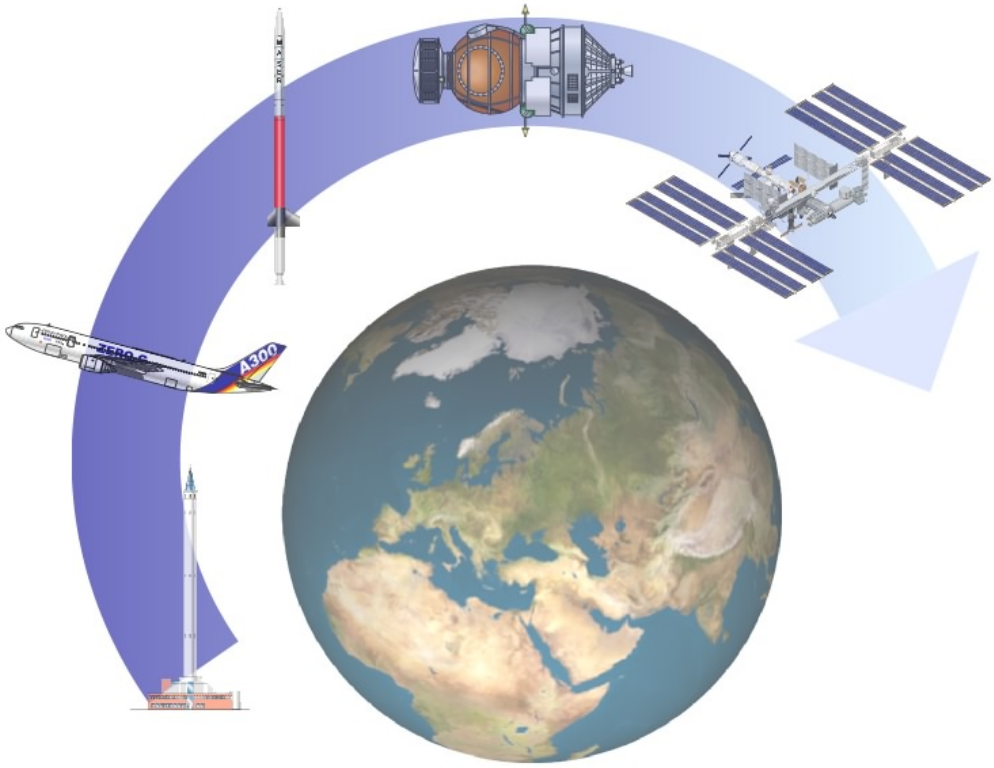
Summary Review of the European Space Agency's Low Gravity Experiments

Volume 2: ISS Increment 8



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PURPOSE OF DOCUMENT

The Summary Review of the European Space Agency's Low Gravity Experiments is intended to provide a concise, but clear, overview of the objectives and scientific results obtained from ESA sponsored low gravity research, executed on/in the five low gravity platforms and other ground based facilities supported by ESA.

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1 INTRODUCTION

1.1 Background to ESA Low Gravity Research

European involvement in low gravity research began approximately 30 years ago, with nationally funded programmes (in particular those of France and Germany) and US collaborations. Later, in January 1982, a European Space Agency (ESA) funded programme was initiated by the ESA Member States, who agreed to a small programme to which governments could contribute according to their interests and budgets. The first phase of this new ESA programme (Microgravity Programme: Phase-1) was established for the period 1982-1985. This allowed ESA to participate in the German Texus Sounding Rocket programme (later extended to include Swedish Maser Sounding Rockets) to perform short duration microgravity experiments. The Phase-1 programme also covered the development of a first set of multi-user experiment facilities to be flown on the Space Shuttle Spacelab and SpaceHab missions.

Since then, ESA has sponsored more than 1500 experiments, payloads and facilities, which have been integrated and operated on various types of low gravity platforms, including:

- Drop Towers;
- Parabolic Flights;
- Sounding Rockets;
- Retrievable Capsules;
- Space Shuttle;
- MIR Space Station;
- International Space Station.

1.2 The Five Major Low Gravity Platforms

This document mainly covers the research executed on/in the 5 major low gravity platforms currently supported by ESA, which are:

- the ZARM (Zentrum für Angewandte Raumfahrt Microgravitation) Drop Tower, located in Bremen, Germany, which was officially declared an ESA External Facility on 2 October 2003;
- the Novespace Airbus A-300 "Zero-g" aircraft based at the Bordeaux-Mérignac airport, which has been used by ESA since 1997;
- the four ESA supported sounding rockets (miniTexus, Texus, Maser and Maxus), which are launched from the Esrange base near Kiruna, Sweden;
- the Russian Foton retrievable capsule, an unmanned Earth-orbiting spacecraft offering microgravity and space exposure, that ESA has used since the early 1990's;
- the most complex platform currently accessible through ESA, the International Space Station (ISS).

Besides the five major low-gravity platforms presented above, ESA also supports access to specific facilities and environments on Earth that simulate low gravity and the confinement of long duration space missions. Extensive and timely use of the research capabilities offered by these facilities, will not only improve the preparation of spaceflight experiments, but will also increase the level of scientific knowledge of the influence of gravity and/or extraterrestrial environments on life, physical and interdisciplinary processes.

Specific ground facilities that simulate space and planetary conditions like climate, physical and psychological isolation, low gravity, extreme environments, high velocity impacts, etc., are available in a wide range of scientific disciplines. Recent examples of these are Long Term Bed Rest Studies (refer to the following web site http://www.spaceflight.esa.int/users/file.cfm?filename=miss-gbfac) and Antarctic Isolation Studies (see http://www.esa.int/esaCP/SEMOS4T1VED_index_0.html). Both types of studies are aimed at investigating the physiological and psychological problems that may arise in conditions of isolation and confinement, such as those that will be experienced during a long duration space mission.

More detailed information regarding the above-mentioned platforms/facilities and how to access them can be found in the ESA publication "European Users Guide to Low Gravity Platforms", which can be viewed at the following web site http://www.spaceflight.esa.int/guide. A hard copy of the Users Guide can also be requested from:

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1.3 Release and Structure of Summary Review Document

This Summary Review document will be released in separate volumes, where each individual volume will cover the research carried out during one or more campaigns (Drop Tower, Parabolic Flight, Sounding Rocket, Ground-based), missions (Foton) or increments (International Space Station). The document will be comprised of two main parts:

- Section 1 will provide general information and a background to ESA's low gravity research, including a summary of the Research Cornerstones.
- Section 2 and beyond will introduce the platform or facility being covered, before providing an experiment-by-experiment summary, broken down per research cornerstone, for each specific campaign, mission or increment.

1.4 Research Cornerstones

In 2000, ESA prepared a comprehensive Research Plan defining the scientific priorities in the life and physical sciences for a 5-year period, with a horizon of 10 years. The compilation of this Research Plan was initiated by a bottom-up analysis of all the research proposals received at that time by ESA. As a next step, ESA asked the European Science Foundation (ESF) to assess the research priorities in a dedicated user consultation meeting, which took place in Bischenberg, France in November 2000. At this meeting and in the subsequent ESF recommendations, the concept of Research Cornerstones was defined.

The Research Cornerstones describe areas of research where concerted efforts at the European level have already produced, or are promising to lead to, eminence if not a leading position on a global level. They provide therefore, an excellent basis for ensuring that new proposals will address issues that have been recognised as constituting a particular strength in Europe. A particular advantage of this will be that the research objectives of the ESA programme will be better harmonised with those of other research funding agencies or entities in Europe, leading to a more efficient and complete coverage of the research efforts involved. It will also further promote the teaming of research groups at European level, thus combining strengths and increasing European knowledge and competitiveness. Finally, it will allow ESA to streamline and optimise the available and future research infrastructure to sustain those objectives.

Already at Bischenberg it was identified that the Research Plan is by definition a living document. Research priorities may shift, new promising research fields may emerge, or new results taken into account. For that reason, it was envisaged that the process of user consultation should be repeated at regular intervals.

Following this, a second user consultation on Life and Physical Sciences in Space was organised again by ESF at Obernai, France in May 2004. On this occasion a larger number of scientists participated and more time was available to discuss the individual disciplines during two workshops. After this consultation ESF recommended

updated Research Cornerstones, which ESA and its advisory committees analysed. After a full investigation, ESA produced an updated Research Plan, in which also the new Research Cornerstones were defined.

It should be stressed, however, that the Research Cornerstones are **not** used as a selection criterion in the evaluation of research proposals. In other words, the final selection of projects is based on scientific quality, regardless of the research topic addressed. This, in the view of ESA, is the only way to ensure that promising new research is identified and pursued. The Research Cornerstones should therefore be seen as a guideline to potential users who wish to carry out research in the life and physical sciences on the ISS.

1.4.1 Life and Physical Sciences Research Cornerstones

The following tables summarise the updated Life and Physical Sciences Research Cornerstones defined in 2004 for the period 2005-2009.

Table 1-1: Fluid Physics Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Fluid and Interface Physics	Study of multiphase systems (their phase transitions and related dynamics), critical and supercritical fluids, granular materials, liquid-solid interface phenomena and complex fluid phases. Geophysical fluid flows.	Quantify heat transfer, mass exchange and chemical processes in multiphase systems and supercritical fluids; Measure diffusive processes in mixtures; Study the stability of foams and emulsions; Describe dynamic coupling in granular materials under vibration.	Develop reactors for supercritical oxidation of industrial contaminants; Develop high-efficiency heat exchangers; Improve reactor design in industrial plants; Design improved oil recovery techniques.
Combustion	Study combustion phenomena that are dominated on the ground by buoyancy convection.	Quantify fuel droplet and spray evaporation, autoignition and combustion processes; Detail the process of soot formation in flames and the conditions for flammability of solid fuels.	Improve efficiency of electrical power plants; Reduce emissions of engines; Fuel-efficient and safe spacecraft for human exploration; Improved flammability test procedures.

Table 1-2: Fundamental Physics Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Physics of Plasmas and Solid/Liquid Dust Particles	Understand the three dimensional behaviour of particles in complex plasmas and aggregation processes that require weightlessness.	Enhance theoretical description of complex plasmas, including self-ordering and phase transition phenomena; Improve modelling of the interaction of protoplanetesimals, their optical properties and of the behaviour of pollutants in the atmosphere.	Develop novel plasma coating techniques; Nucleation and growth of novel substances for solar cells and plasma screens; Improved modelling of Earth climate and environment.
Cold Atom Clocks, Matter Wave Interferometers and Bose-Einstein Condensates	Study properties and applications of cold atoms, including Bose-Einstein condensates.	Develop and operate a cold atom clock in space; Check limits of validity of theories of relativity and quantum electrodynamics.	Improved accuracy of absolute time measurements; Increased accuracy for navigation and geodesy systems.

Table 1-3: Material Sciences Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Thermophysical Properties of Fluids for Advanced Processes	Utilise the extended possibilities of containerless processing in space to measure critical properties of fluids for processes that are required as input parameters for adequately describing balances in volume phases and at interfaces.	High accuracy measurements of the properties of stable and metastable (undercooled) liquid metals.	Increase the reliability of numerical simulation and control of casting facilities in the metallurgical industry.
New Materials, Products and Processes	Understand the physics of solidification and crystal growth of metals, organic and inorganic materials and biological macromolecules.	Quantify the influence of the growth conditions on the homogeneity and the defects in crystals, including protein crystals; Enhance numerical models of the microstructure formation in metals and alloys.	Improve and validate models for predicting grain structures in industrial castings; Develop processes towards new metallurgical products; Improve efficiency of production of industrial crystals.

Table 1-4: Biology Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Molecular and Cell Biology	Study the impact of gravity at the cellular and molecular levels.	Study gene expression in an altered gravitational environment in relation to cellular phenomena; Improve understanding of the impact of gravity on signal transduction and the specific properties of cellular entities such as the membrane; Clarification of the role of mechanical forces including those derived from gravity in triggering proliferation, differentiation, apoptotic processes and tissue formation.	Provides the basis for other disciplines, including developmental biology, physiology, health science and biotechnology; Develop artificial functional tissues and targets for drugs screening; Depression of the immune system; Identify pharmacological substances for tissue regeneration; Develop bio-regenerative life support systems for human exploration missions; Develop novel microencapsulated drugs and cells.
Plant Biology	Understanding the impact of gravity on plant systems; Study mechanosensory elements involved in mechanisms of graviorientation and gravishaping.	Identify molecular and cellular elements of mechanosensory mechanisms and gravity-related signalling pathways; Study how gravity shapes plant morphology; Identify gene interactions important in the gravistimulus response chain.	Improvement of plant growth and mechanical properties of plants; Develop and improve biological life support systems; Provide the basis for biotechnological applications utilised on future long-term human spaceflight; Develop techniques for plant survival and growth in space.
Developmental Biology	Study the effect of gravity on whole-body developmental and reproductive processes.	Study altered gene expression in an altered gravitational environment; Study the impact of the cytoskeleton architecture on signal transduction e.g. functional genomics; Identify gravity-sensitive phases in multicellular organisms; Understand the effect of gravity on the development of the vestibular and sensori-motor systems in vertebrates.	Design pharmacological relevant substances for animal and human applications relevant to human development; Evaluation of the possible outcome of extraterrestrial colonisation attempts; Develop techniques and pharmacological substances for tissue regeneration.

Table 1-5: Physiology Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Integrative Gravitational Physiology	Explore, in an interdisciplinary way, systems that are sensitive to gravity, e.g. cardiovascular system, pulmonary system, nervous system, fluid-electrolyte homeostasis, skeletal system, immune system, etc.	Study cardiovascular control and regulation; Study the mechanisms for fluid regulation by the kidneys; Investigate the interaction of the vestibular system with other inputs relevant to locomotion and posture (e.g. vision, proprioception); Study effects of changes in load on muscle atrophy and plasticity; Understand and quantify bone mass turnover as a function of e.g. local blood perfusion and mechanical stress; Study the mechanisms of osteoporosis.	Improve techniques and devices for medical applications e.g. sports medicine; Improve rehabilitation after long-term incapacitation, particularly involving bed rest; Improve treatment of patients with decreased lung-function; Develop improved approaches for the treatment of neurological diseases; Improve means for diagnostics, prevention and treatment of osteoporosis, and reduce bone loss in astronauts for future long duration missions; Improve treatment of diseases like hypertension.
Non-Gravitational Physiology of Spaceflight	Explore the effects of the non-gravitational extreme environment of space, e.g. radiation, isolation, nutrition, confinement, noise, disruption of circadian rhythms, hypobaric conditions (e.g. EVA), etc.	Study effects of isolation, group dynamics, cultural differences, etc.; Study effects of radiation on DNA damage; Study close coupling between nutrition and health, e.g. testing new space foods; Investigate effects of dust inhalation on airway inflammation; Investigate possibilities of decompression sickness in connection with EVA.	Improve crew selection techniques for future long duration missions; Develop new nutritional methods for the improvement of health; Develop new protection measures for people exposed to radiation; Improve prevention and treatment for patients suffering from decompression sickness.
Countermeasures	Develop physiological, pharmacological, psychological, and mechanical countermeasures.	Understand the mechanisms leading to various problems such as: spatial disorientation (nausea, imbalance), orthostatic intolerance, bone loss and microarchitectural deterioration, muscle atrophy and weakness, cardiac atrophy, etc.	Develop improved approaches, treatment and countermeasures for a variety of Earth and space based disorders and maladies.

Table 1-6: Exobiology Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Origin, Evolution and Distribution of Life	Study the survivability of organisms under extreme conditions on Earth (extremophiles) and in space.	Investigate the contribution of space conditions, including radiation, to the formation of prebiotic molecules; Identify the conditions for survivability of micro-organisms from and in space, including planetary surfaces; Identify markers and tools to search for extinct and extant life.	Identify novel enzymes and bacteria from extreme physical and chemical environments with industrial application e.g. biocatalysis.

Table 1-7: Exploration Research Cornerstones

RESEARCH CORNERSTONES	DESCRIPTION	SCIENCE TARGETS	POTENTIAL APPLICATIONS
Human Planetary Exploration	Study novel aspect of human planetary expeditions.	Quantify radiation risk for human beings and understand the specific biological action of space radiation; Study effects of isolation in high-stress environments; Quantify needs for consumables during missions; Perform simulation tests on in-situ resource utilisation potential.	Develop advanced radiation sensors and countermeasure devices; Develop technology for telemedicine/telesurgery in remote areas; Develop protocols for handling stress effects; Develop methods for in-situ resource utilisation; Develop life-support systems for use in space and other isolated environments; Develop the technologies for identification and utilisation of in-situ resources.

For more details regarding Life and Physical Sciences research, please contact:

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1.5 Erasmus Experiment Archive (EEA)

An important resource for low gravity research scientists and users is the Erasmus Experiment Archive (EEA), maintained by the Erasmus Centre (HME-UC). The EEA is a database of ESA funded or co-funded experiments covering a wide range of scientific areas, which were performed during missions and campaigns on/in various space platforms and microgravity ground-based facilities over the past 30 years. The archive is continuously being updated and as of February 2007, contained more than 1900 experiment records. The major items of information covered in the EEA include:

- Research cornerstone;
- Date of experiment;
- Mission name;
- Team members and institutes;
- List of publications/references;
- Experiment objectives;
- Experiment procedures;
- Experiment results;
- Attachments (figures, graphs, videos, etc.).

The EEA depends highly on the support provided by users; therefore users are encouraged to send inputs to the contact coordinates below, once they have executed an experiment. In fact, users who perform ESA funded experiments have the obligation to provide an abstract to the EEA. Failure to meet this obligation will be taken into account when deciding on new experiment opportunities/proposals from the user team in question.
Users are invited to visit the database, from which they can, among other things, obtain further information regarding experiments of their field of research already carried out in the past. The EEA web address is the following: http://www.spaceflight.esa.int/eea. For further details regarding the EEA, please contact the following by phone, fax, mail or e-mail:

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1.6 General Information and Advice

Any comments, suggestions or requests for further information regarding the ESA low gravity research programme, should be sent to one of the following by phone, fax, mail or e-mail:

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2 THE INTERNATIONAL SPACE STATION (ISS)

2.1 ESA Utilisation Rights and Additional Flight Opportunities

The National Aeronautics and Space Administration (NASA) provides the overall leadership of the ISS programme development and implementation, and together with Russia provides the major building blocks of the ISS. The European Space Agency (ESA), together with the Japan Aerospace Exploration Agency (JAXA) and the Canadian Space Agency (CSA) are providing additional elements, which significantly enhance the Space Station. The overall ISS utilisation rights are divided among the Partners, according to the elements and infrastructure they provide (e.g. Columbus Laboratory for ESA). The main principle is that each International Partner may utilise equipment and facilities in or on each other Partner's elements in accordance with their respective "utilisation rights". Those rights are defined in the Intergovernmental Agreement (Article 9) and the different Memoranda of Understanding signed by all of the Partners.

In return for its contribution to the ISS, ESA has a resource allocation of 51 % of the internal and external user accommodation of the Columbus Laboratory. Other allocation rights to ESA comprise 8.3 % of the total ISS utilisation resources and 8.3 % of the total crew time. Note that this excludes all of the Russian accommodations and resources, as this is retained by Russia for its own use.

In May 2001, ESA and the then Russian Aviation and Space Agency (Rosaviakosmos), now Roscosmos, signed a Framework Agreement for the provision of Russian ISS flight opportunities. The Agreement documents the principles, terms and conditions for the cooperation between ESA and Roscosmos concerning ISS operations and utilisation, through the provision by the latter of fare-paying ISS flight opportunities in the period 2001-2006, for members of the European Astronaut Corps. The actual commitment for a specific flight opportunity is entered by ESA upon signature of an ISS Flight Order Contract (IFOC) for a specific flight.
The Framework Agreement, establishes a solid and stable basis for the strategic planning of the European Astronaut Corps, and it represents an important step towards the further development of operational expertise of the ESA astronauts prior to the full European utilisation of the ISS with the launch of Columbus.

Two types of flight opportunities are considered under the Agreement as ISS flight opportunities:

- ISS "taxi flights" (this term is reported in the original agreement, but is no longer used), which are defined as short duration Soyuz flights to the ISS for the purpose of exchanging the ISS docked Soyuz, including a short duration stay (approximately 7-8 days) on-board the ISS;
- ISS increment flights, which are defined as ISS crew exchange flights, including a 3-6 months (one increment) stay on-board the ISS.

The assignment of back-up astronauts/cosmonauts for ISS flight opportunities, involving ESA astronauts, is agreed upon between ESA and Roscosmos for each flight.
On-board activities are not restricted to the mandatory system operations and maintenance activities, but also allow for the conduct of activities or experimental programmes in the interest of ESA and national organisations of the ESA Member States. The terms and conditions of such activities are agreed upon in each specific IFOC. The IFOC defines the terms and conditions specific to the implementation of an agreed ISS flight opportunity. Such terms and conditions take precedence over the terms and conditions defined in the Framework Agreement.

The following table (Table 2-1) summarises the Russian ISS flight opportunities that have thus far included an ESA astronaut on-board, following the signature of the Framework Agreement in May 2001.

Table 2-1: ESA Russian flight opportunities deriving from ESA/Roscosmos Framework Agreement (May 2001)

ISS MISSION	ESA MISSION NAME	VEHICLE ID	LAUNCH DATE	LANDING DATE	ESA ASTRONAUT	ASTRONAUT NATIONALITY
ISS 3S	Andromede	Soyuz TM-33	21/10/2001	31/10/2001	Claudie Haigneré	French
ISS 4S	Marco Polo	Soyuz TM-34	25/04/2002	05/05/2002	Roberto Vittori	Italian
ISS 5S	Odissea	Soyuz TMA-1	30/10/2002	10/11/2002	Frank De Winne	Belgian
ISS 7S	Cervantes	Soyuz TMA-3	18/10/2003	28/10/2003	Pedro Duque	Spanish
ISS 8S	DELTA	Soyuz TMA-4	19/04/2004	30/04/2004	Andre Kuipers	Dutch
ISS 10S	Eneide	Soyuz TMA-6	15/04/2005	25/04/2005	Roberto Vittori	Italian
ISS ULF1.1	Astrolab	Shuttle STS-121	04/07/2006	22/12/2006	Thomas Reiter	German

2.2 Increment Timeline

The summary review of experiments carried out on board the ISS will be presented per Increment, i.e. the period of time between the launch of a vehicle carrying an exchange crew to the ISS, and the undocking of a vehicle for return of that crew. The length of an increment ranges anywhere from 3 months to about 6 months.
The Summary Reviews of European ISS experiments will be covered as from the Belgian Soyuz Mission ("Odissea"), i.e. as from the end of Increment 5.
The following schematic (Figure 2-1) presents a basic timeline of launch events and Increments of the ISS programme, and serves as a quick reference for users of this document.

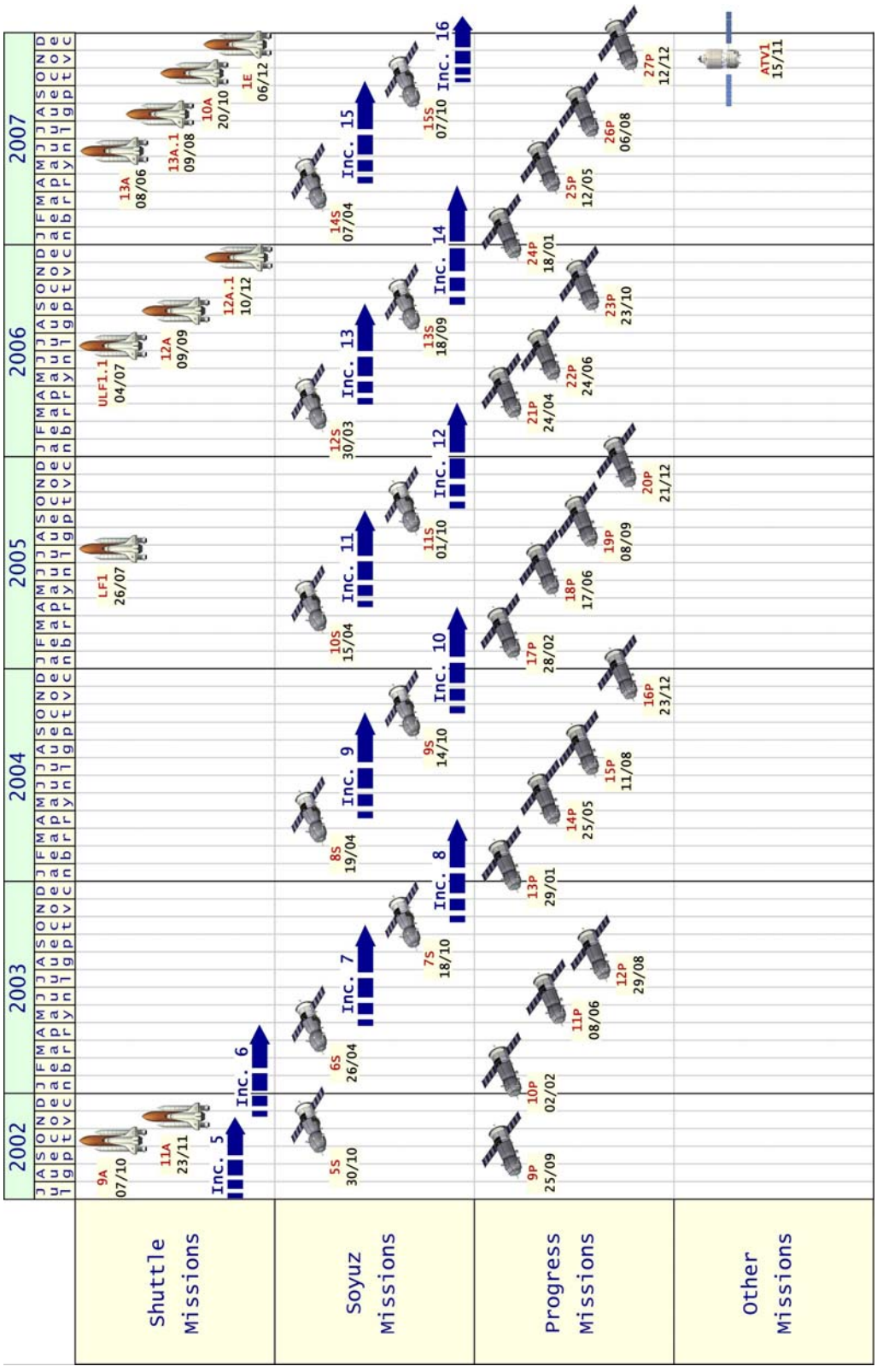

Figure 2-1: ISS Programme Launch Events and Increments (July 2002 - December 2007)

2.3 Increment 8: ESA experiments

The 8 ESA experiments carried out during Increment 8 formed part of a larger scientific programme (24 experiments) that was developed for the Spanish Soyuz Mission, "Cervantes", launched on October 18th, 2003, carrying the Spanish ESA astronaut Pedro Duque to the ISS for a 7-day stay on the Station.

The following table (Table 2-2) lists the 8 ESA experiments that will be covered by this report.

Table 2-2: List of ESA experiments for Increment 8

NAME OF EXPERIMENT	RESEARCH CORNERSTONE
LIFE SCIENCES	
Chromosomal aberrations in blood lymphocytes of astronauts **(CHROMOSOME-1)**	Biology: Molecular and cell biology
Microbial experiment on Space Station about gene expression **(MESSAGE-2)**	Biology: Molecular and cell biology
Effects of the gravity altered environment on Drosophilia motility, behaviour and ageing **(AGEING)**	Biology: Developmental biology
Directed attention brain potentials in virtual 3-D space in weightlessness **(NeuroCOG)**	Physiology: Integrative gravitational physiology
Cardiovascular adaptation to weightlessness **(CARDIOCOG-1)**	Physiology: Integrative gravitational physiology
Sympathoadrenal activity in humans during spaceflight and bed rest **(SYMPATHO-1)**	Physiology: Integrative gravitational physiology
PHYSICAL SCIENCES	
Counterdiffusion protein crystallisation in microgravity and its observation with the Protein Microscope for the International Space Station **(PromISS-2)**	Material Sciences: New materials, products and processes
Study of aggregation mechanism and kinetics of ZSM-5 and Silicalite-1 nanoslabs into ZSM-5/Silicalite-1 hybrid phases under microgravity conditions (**NANOSLAB**)	Material Sciences: New materials, products and processes

2.3.1 Life Sciences

2.3.1.1 Biology: Molecular and cell biology

2.3.1.1.1 Chromosomal aberrations in blood lymphocytes of astronauts (CHROMOSOME-1)

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2.3.1.1.1.1 Background, Objectives and Procedures

Cosmic radiation is a major risk factor in human space missions. During space flights astronauts are chronically exposed to radiations of solar and galactic origin. The space radiation field consists of electrons, protons, heavy particles, and secondary radiation like bremsstrahlung, neutrons, and charged particles created by interactions of primary radiations with nuclei of spacecraft shielding material or the human body. The contribution of the dose of single radiation types depends on altitude and inclination of the spacecraft, effective shielding thickness and solar activity during the mission. Although it can be assumed that radiation plays a major role in mutation induction in astronauts, synergistic influences such as weightlessness, acceleration, vibration hyperthermia, noise microwave radiation, physical exercises, trauma, and infections cannot be ruled out.

The objective of this experiment was to study chromosomal aberrations in human blood lymphocytes to assess the mutagenic potential of space radiation in man. It is hypothesised that cosmic radiation induces chromosomal aberrations in space flight crew members. This effect should depend on the radiation dose received, e.g. short flight crew members are if at all less affected than long duration crew members. The association of chromosomal aberrations with an enhanced cancer risk stresses the importance of the planned research. The data obtained will be helpful in order to carefully plan space flight missions.
In the CHROMOSOME-1 experiment, 4 astronauts on ESA missions to the ISS were analysed for the induction of chromosomal aberrations during the flight. The flight durations ranged from 10 to 11 days. CHROMOSOME-1 was conducted as part of the experimental package of the ESA supported Belgian Soyuz mission, "Odissea" (ISS 5S mission), which took place in October-November 2002, and the Spanish Soyuz mission, "Cervantes" (ISS 7S mission), which was executed in October 2003, during increments 5 and 8 respectively. CHROMOSOME-1 was also carried out during increments 6-11 with NASA support. For more information about the NASA supported increments regarding CHROMOSOME-1 please go to:
http://exploration.nasa.gov/programs/station/Chromosome.html
To assess the genetic impact of the radiations, 10 ml of blood was drawn a few days before launch and directly after flight (R+1) by venous puncture. Whole blood cultures were set up with phytohemagglutinin to stimulate lymphocytes to enter the cell cycle. Forty-eight hours after start of incubation, chromosome preparations were performed using colchicine to arrest mitotic metaphase stages. Three different staining procedures were performed to assess all types of aberrations induced by ionising radiations:

1. Classical Giemsa block-staining to score dicentric and ring chromosomes, and excess fragments in the whole chromosome set (Figure 2-2):
2. 24 colour fluorescence in-situ hybridisation (FISH) to score reciprocal translocations and complex aberrations involving two or more chromosomes with at least three breaks (Figure 2-3);
3. Multi-colour high resolution banding FISH of chromosome 5 to score for inversions and translocations between homologous chromosomes (Figure 2-4).

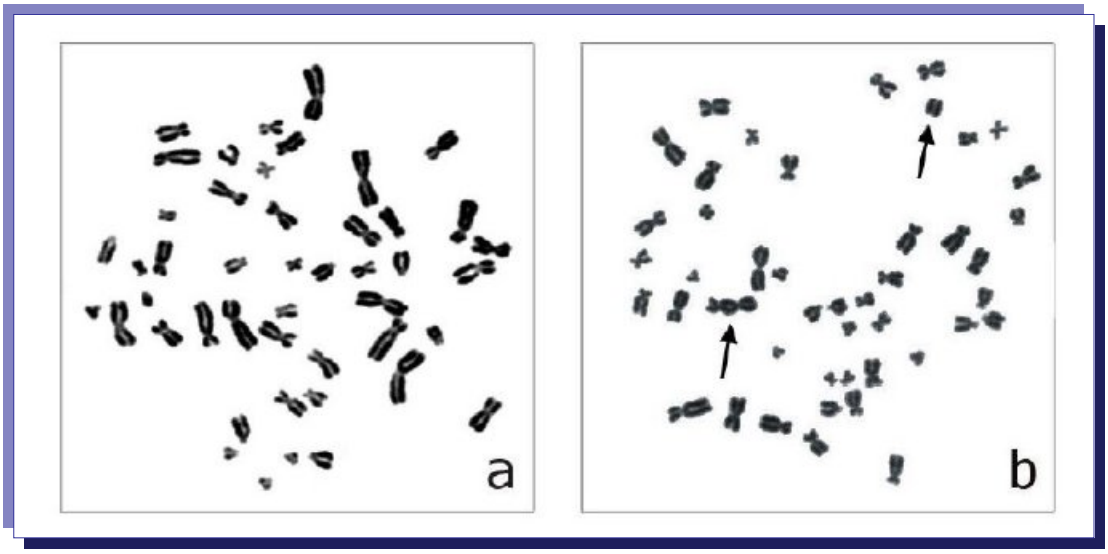

Figure 2-2: A normal set of 46 chromosomes prepared from a cultured human peripheral lymphocyte following Giemsa-staining (a) and a metaphase showing a radiation-induced dicentric chromosome with an associated fragment (b).

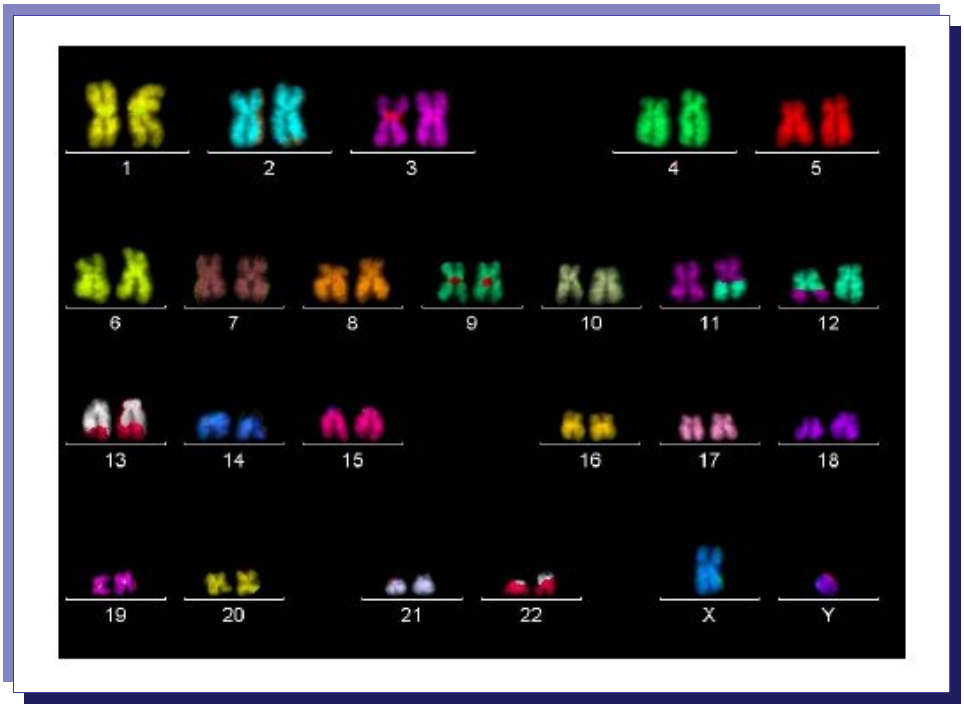

Figure 2-3: Human karyotype following 24 colours in situ hybridisation (mFISH) with a reciprocal translocation between chromosome 11 and 12.

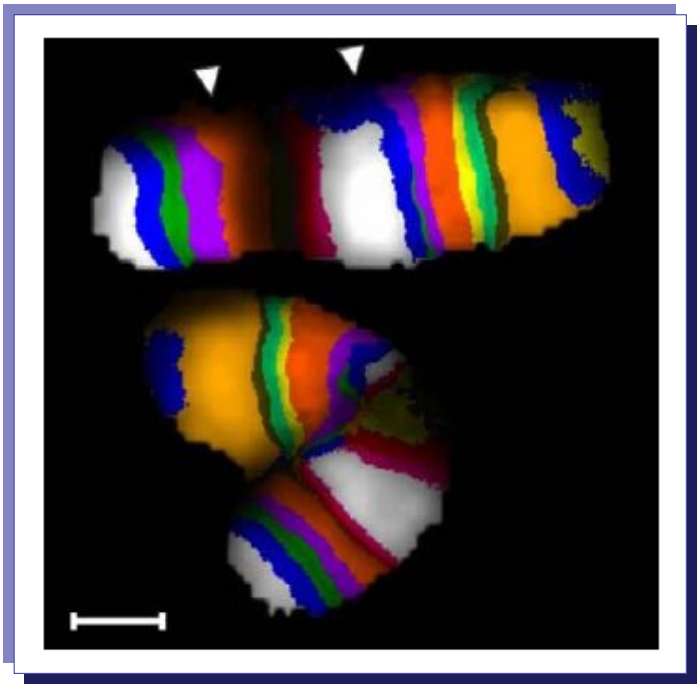

Figure 2-4: Human chromosome 5 following banding FISH (mBAND) showing an inversion in the lower chromosome. The inverted segment is indicated by arrows in the normal upper chromosome 5.

A quantitative comparison between pre- and post flight aberration values will provide information about chromosome breaking effects of cosmic radiation in blood lymphocytes of astronauts correlated with flight duration and extra vehicular activity (EVA) duration. In the ESA missions presented here the mutation rates from 4 short duration mission astronauts were determined. The results from the study were compared with results obtained from crew members of long duration missions.

2.3.1.1.1.2 Results

CHROMOSOME-1 was carried out during both the Belgian Soyuz mission, "Odissea" and the Spanish Soyuz mission, "Cervantes". The lymphocyte cultures from the blood samples of the 4 short duration crew members were grown well and gave good preparations for scoring of chromosomal aberrations.
The analyses of the blood samples of the 4 short duration crew members of the two missions mentioned above revealed no overall increase of aberrations. In Figure 2-5 the mean percentage of aberrant cells before and after flight is shown. For comparison, the mean values of 11 long duration flight crew members are included. While the increase of mutated cells by a factor of 1.4 was significant for the latter group no such increase was found for the 4 short duration subjects.

The detailed results of all assays employed are shown in Table 2-3. Before the flights only chromosomal fragments were detected (all values in control range). After the flight few dicentrics could be observed. No other aberration types which are considered to be radiation induced were found. These results are in contrast with the observations in long duration crew members (data not shown). Following missions of approximately 6-month periods, various types of aberrations increased after return to Earth.

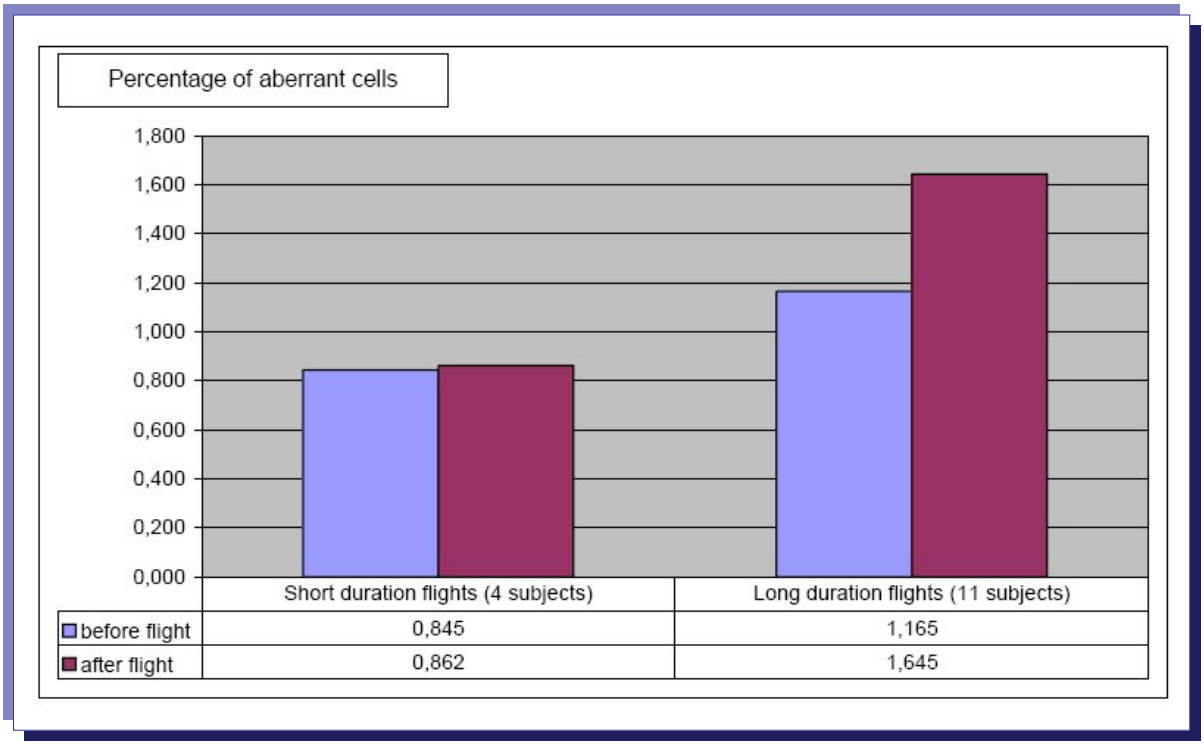

Figure 2-5: Mean values of aberrant metaphases in the 4 ESA mission astronauts and the respective values for the long duration ISS crew members. Data are compiled from all stainings.

Table 2-3: Results of all assays employed before and after flight.

BEFORE FLIGHT					
Astronaut Code Number	**Total number of metaphases analysed (all assays)**	**Dicentric chromosomes (all assays)**	**Fragments (all assays)**	**Reciprocal translocations (mFISH/mBAND)**	**Intrachromosomal aberrations (mBAND)**
0	773	0	7	0	n.d.
1	1928	0	5	0	n.d.
2	2543	0	5	0	n.d.
3	2419	0	9	0	0
Total	**7663**	**0**	**26**	**0**	**0**
AFTER FLIGHT					
Astronaut Code Number	**Total number of metaphases analysed (all assays)**	**Dicentric chromosomes (all assays)**	**Fragments (all assays)**	**Reciprocal translocations (mFISH/mBAND)**	**Intrachromosomal aberrations (mBAND)**
0	952	1	0	0	n.d.
1	1842	0	5	0	n.d.
2	1181	1	5	0	n.d.
3	1244	2	7	0	0
Total	**5219**	**4**	**17**	**0**	**0**

2.3.1.1.1.3 Conclusions and Recommendations

The compilation of results obtained in the ESA missions (and future short duration Soyuz missions) and of long term missions is expected to provide almost complete information about chromosomal aberrations in peripheral lymphocytes of crew members exposed to space radiation. The results will enable a better assessment of the genetic risk of humans in space and in consequence, will help to optimise radiation shielding. The data will also allow calculations of aberration frequencies expected during deep-space missions.

2.3.1.1.1.4 Publications

1. M. Horstmann, M. Durante, C. Johannes, R. Pieper, G. Obe, (2005), "Space radiation does not induce a significant increase of intrachromosomal exchanges in astronauts' lymphocytes", *Radiat Environ Biophys Vol. 44, Issue 3*, pp. 219-224
2. M. Horstmann, M. Durante, C. Johannes, G. Obe, (2005), "Chromosomal intrachanges induced by swift iron ions", *Adv Space Res Vol. 35, Issue 2*, pp. 276-279
3. M. Horstmann, M. Durante, G. Obe, (2004), "Distribution of breakpoints and fragment sizes in human chromosome 5 after heavy-ion bombardment", *Int J Radiat Biol Vol. 80, Issue 6*, pp. 437-443
4. M. Durante, K. Ando, Y. Furusawa, G. Obe, K. George, F.A. Cucinotta, (2004), "Complex chromosomal rearrangements induced in vivo by heavy ions", *Cytogenet Genome Res Vol. 104, Issue 1-4*, pp. 240-244
5. C. Johannes, M. Horstmann, M. Durante, I. Chudoba, G. Obe, (2004), "Chromosome intrachanges and interchanges detected by multicolour banding in lymphocytes: Searching for clastogen signatures in the human genome", *Radiat Res Vol. 161, Issue 5*, pp. 540-548
6. M. Durante, G. Snigiryova, E. Akaeva, A. Bogomazova, S. Druzhinin, B. Fedorenko, O. Greco, N. Novitskaya, A. Rubanovich, V. Shevchenko, U. von Recklinghausen, G. Obe, (2003), "Chromosome aberration dosimetry in cosmonauts after single or multiple space flights", *Cytogenet Genome Res Vol. 103, Issue 1-2*, pp. 40-46
7. O. Greco, M. Durante, G. Gialanella, G. Grossi, M. Pugliese, P. Scampoli, G. Snigiryova, G. Obe, (2003), "Biological dosimetry in Russian and Italian astronauts", *Adv Space Res Vol. 31, Issue 6*, pp. 1495-1503

2.3.1.1.2 Microbial experiment on Space Station about gene expression (MESSAGE-2)

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2.3.1.1.2.1 Background, Objectives and Procedures

The early detection of changes in single bacterial cells and bacterial communities that are present in spacecraft is crucial for a variety of biosafety issues (i.e. pathogenicity for crew, biodegradation of materials, etc.). Bacteria will also be essential for long-term manned missions (e.g. to Mars) for the recycling of waste and the production of food.
The main objective of the MESSAGE experiments was to study the effects of space conditions such as weightlessness and cosmic radiation on board the ISS on physiological and metabolic processes in bacteria. The MESSAGE experiments studied many different aspects of bacterial activity using many different microbial and molecular methods. The effects of space conditions on the cell motility by means of flagella, the cell physiology, the cell metabolism, and the genetic stability and rearrangements in bacteria was studied on the cell level and on the molecular level. Through the molecular analysis of the transcriptome and proteome special attention was given to the activation or inactivation of genes involved in the response to stress. This approach led to a unique view on the total physiological and metabolical response of a whole organism to a specific growth condition as space.
The first MESSAGE experiment (MESSAGE-1) was performed by the Belgian astronaut Frank De Winne on board of the International Space Station (ISS) during the Belgian Soyuz Mission in November 2002. The second MESSAGE experiment (MESSAGE-2) was essential for reproducibility and statistical analysis of the scientific

results obtained during the first experiment. Scientific conclusions based on MESSAGE 1 and 2 could then be integrated in other microbial space projects.
For the MESSAGE experiments two different well known (genome sequenced) and non-pathogenic bacteria, i.e. *Ralstonia metallidurans* CH34 (currently renamed to *Cupriavidus metallidurans* CH34) and *Rhodosprillum rubrum* S1H, were incubated in the ISS as model organisms. *Ralstonia metallidurans* CH34 (ATCC 43123) is a flexible, versatile and robust well-studied non-pathogenic β-Proteobacterium which can survive in harsh environmental conditions. *Rhodospirillum rubrum* S1H (ATCC25903) is a motile spiral shaped α-Proteobacterium which was not used in the MESSAGE-1 experiments but was added in MESSAGE-2 because of its importance in other ESA-supported research projects and other European labs. *Rhodospirillum rubrum* is colonizing the second bioreactor of the future 'bioregenerative life support system for space exploration' called MELiSSA under development at ESA.
The bacteria were cultivated in 1.5ml liquid medium in hermetically sealed 1.8ml tubes or in drops on semi-solid 1.5 % agar medium in hermetically sealed Petri dishes with a 6cm diameter, filled with 10ml of medium, all individually packed in plastic bags. Culture tubes were packed in a passive cooling dewar container (T) cooled by a frozen cooling tube for upload and in a foam container (R) for download, while Petri dishes were packed in 2 hermetically sealed plastic containers (P1 and P2). In each container a package of temperature and radiation sensors was included.
The MESSAGE-2 experiment space flight hardware was basically commercially available, standard sterile laboratory plastic components (containers/tubes/plates) and commercially available temperature and radiation sensors. All components worked perfect without negative effects on the biological samples or without any leaks or damages. However, the passive cooling container T, made from 'aerogel', was heavy and did not keep the samples sufficiently cool and it is therefore advised not to use the same design and material again in future experiments.

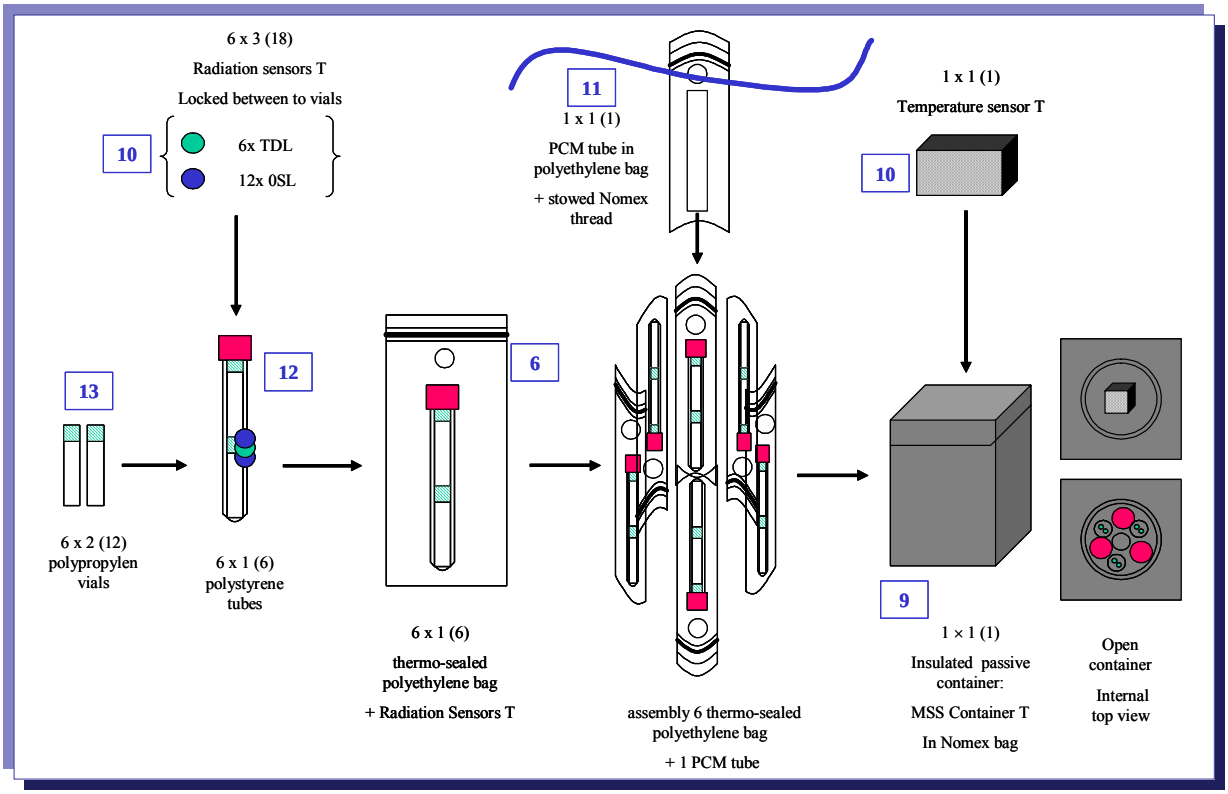

Figure 2-6: Schematic of T container

Pre-launch experiment preparation, assembly and safety control were performed 'on site', *i.e.* in Baikonur in Kazakhstan, to reduce negative effects of pre-flight transport and prolonged cold storage as were experienced during the MESSAGE-1 experiment. The full experiment packages was put in an insulator box with ice packs and handed over for disinfection and integration in the Soyuz vehicle 18 hours before launch. A ground control experiment was inoculated at the same time, transported back to SCK/CEN and maintained at identical conditions

parallel in time. Post-flight a second ground control experiment was run according to the in-flight recorded temperature profile.

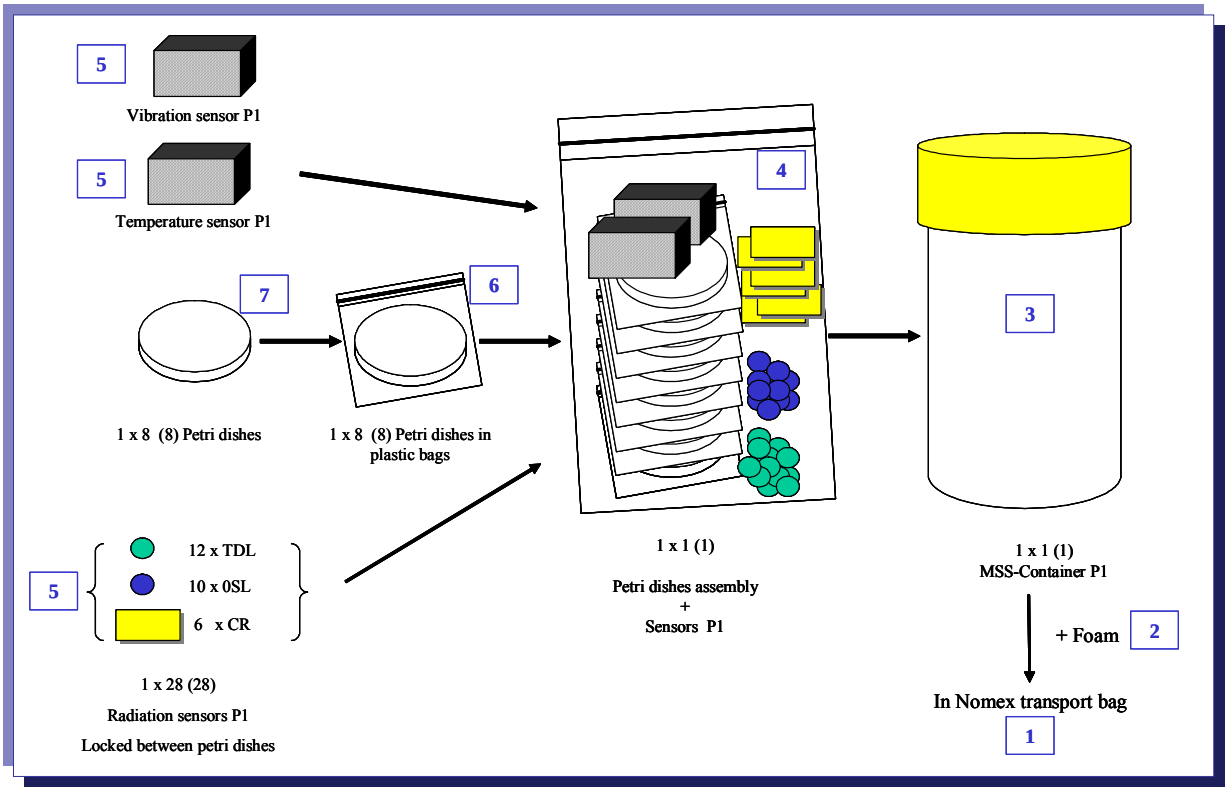

Figure 2-7: Schematic of P1 and P2 containers

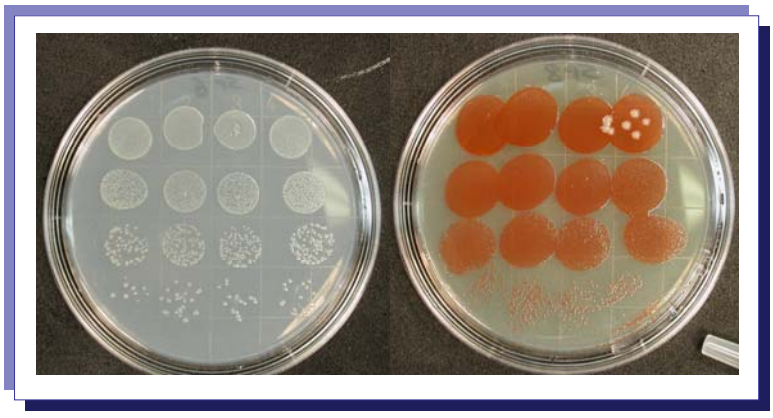

Figure 2-8: Bacterial cultures on agar medium in Petri dishes

During integration and upload in the Soyuz vehicle, the liquid cultures were cooled in a dewar container with a passive cooling ice tube, while the agar cultures were incubated at ambient temperature. Unfortunately the passive cooling was inadequate and temperature went up quickly. Once in space, all the microbial samples were incubated for 7 days in the dark in containers P1, P2 and R at ambient temperature in the Service Module in the Russian segment of the ISS. The temperature data indicated a relative constant temperature of approximately 22°C. The total cumulative radiation dose (sum of low LET and high LET radiation spectrum) recorded in the MESSAGE-2 experiment containers during the 10-day space flight was 1.80 mGy. During the first 6 days in the ISS, one sample of the tube experiment was removed from container R every day and stored in the Kriogem-03 freezer (-20°C) with the ice tube. After the last session the temperature sensor was removed from the container R lid and placed in the freezer with the samples. However, the temperature recording during sample storage in the Kriogem-03 freezer onboard the ISS indicated a constant temperature of -2.0°C instead of the requested -20.0°C. It is still

unclear what caused this temperature difference. At the end of the mission, 12h before undocking of the Soyuz vehicle from the ISS, all frozen tubes, including the cooling tube, were packed again in the foam container R and stowed in the Soyuz for return. The 2 Petri dish experiments returned unopened without freezing. The experiment thus required only limited crew operations.

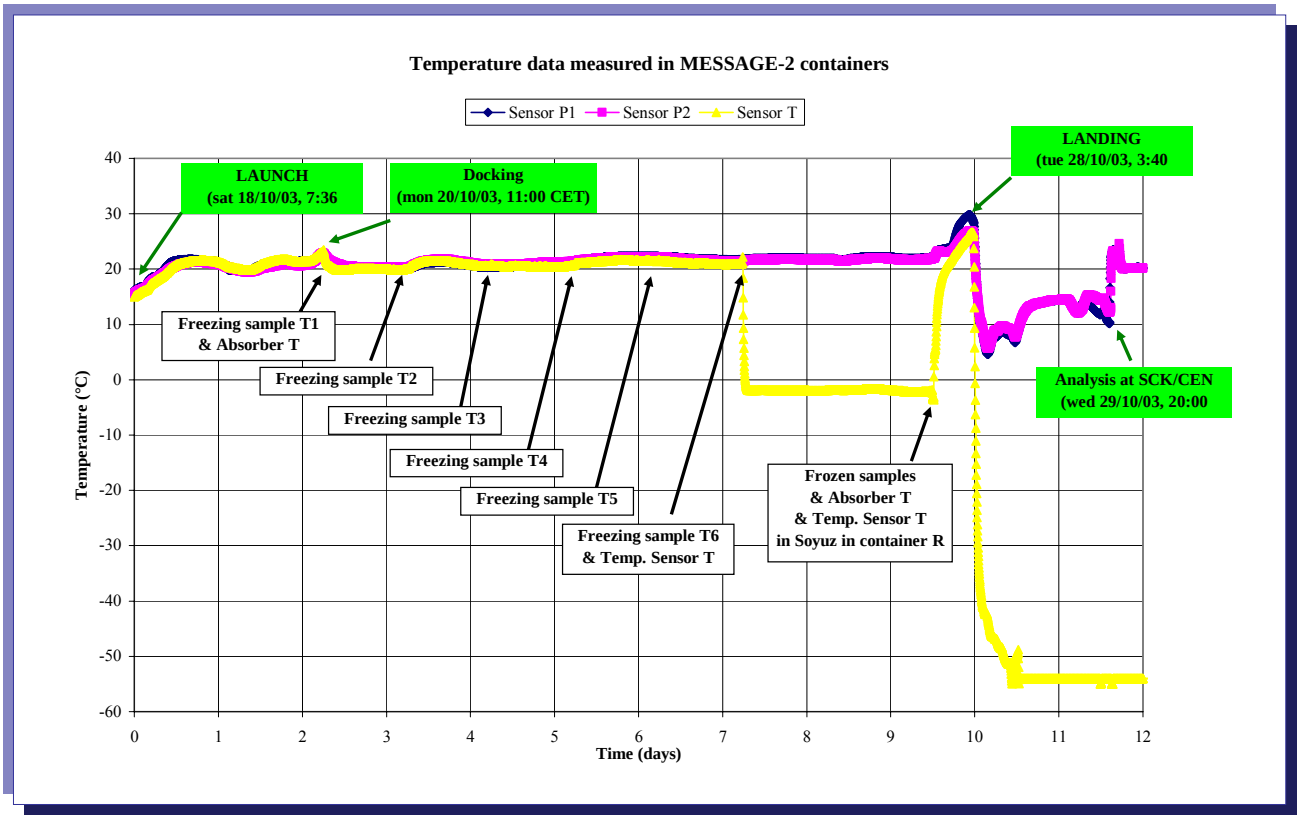

Figure 2-9: MESSAGE 2 experiment temperature recordings

Immediately after landing all samples were transported in dry ice (tubes) or with ice packs (Petri dishes) by ESA members to Amsterdam airport (The Netherlands) and further to the laboratory of the science team in Mol, Belgium. The analysis was started 36 hours after landing. The bacterial survival and growth kinetics were assessed by quantification of colony growth on solid medium and by optical density measurements of liquid cultures. The cell physiology was analyzed by flow cytometry immediately upon retrieval of the samples. In addition, changes in metabolism of *R. rubrum* were tested using replicaplating with colonies harvested from space and ground grown plate cultures, spotted on various media, and incubated under different metabolic conditions.

2.3.1.1.2.2 Results

The differences in bacterial survival and growth observed between space and ground cultures of *R. metallidurans* CH34 in a first MESSAGE-1 experiment were not observed anymore in the second space experiment MESSAGE-2. Also for the bacterium *R. rubrum* S1H cell survival count indicated no significant difference between space and ground grown cultures in the MESSAGE -2 experiment. It should be noted that a more stable temperature control during pre-, in- and post flight in the second space experiment MESSAGE-2 was possible, compared to the first MESSAGE-1 experiment. In the MESSAGE-1 experiment all samples were prepared in ESTEC-ESA in Noordwijk in the Netherlands 4 days before launch and transported to Baikonur in Kazakhstan in an insulator box with ice packs. In the MESSAGE-2 experiment the samples were filled on site and stored on ice only 18 hours before launch. In addition, the incubation temperature in the ISS fluctuated during the MESSAGE-1 experiment but was very constant (circa 22°C) during the MESSAGE-2 experiment. Compared to the storage in the return Soyuz as was done in the MESSAGE-1 experiment, passive storage in the Service Module yielded a much more stable ambient temperature.

The motility of *Ralstonia metallidurans* CH34 in semi-solid agar (0.6%) medium showed no significant differences between ground and space grown cultures. However, the motility test was based on pure visual analysis of swimming and swarming distance and did not allow good quantitative analysis. The design for motility testing in space can be improved for future experiments. No motility tests were performed on *R. rubrum* S1H.
Flow cytometry data did not show any significant difference between *R. metallidurans* CH34 cells grown on agar medium in space flight or ground conditions for cells size and shape, membrane integrity and potential, intracelluar pH, and intracellular concentrations of reactive oxygen species. For *R. rubrum* S1H, significant differences in cell physiology were observed. Space grown cells showed a smaller 'side scatter', indicating a change in cell size and shape in space conditions. Cell permeability (assessed with fluorochrome propidium iodide) was increased and membrane potential (assessed with fluorochrome rhodamine123) was reduced under space conditions, indicating a lower viability of cells grown in space conditions. Interestingly, all chromophores detecting ROS (assessed with fluorochromes hydroethidine, dihydrorodamine) or enzyme activity induced by ROS (assessed with fluorochrome mercury orange) indicated a lower level of oxidative stress under space conditions.
Transcriptomic (RNA) analysis was done using semiquantitative PCR and a full genome DNA gene chips of *R. metallidurans* CH34 and *R. rubrum* S1H. Unfortunately as the cultures grown on agar were 10 days old and already at the end of their lifetime (in stationary phase), only very little RNA of good quality could be retrieved. As such, RNA analysis data did not show significant difference between cells grown in space or on ground. The transcriptomic analysis of *R. rubrum* S1H from the MESSAGE 2 experiment showed 372 genes that were significantly up-regulated under space conditions. Typical genes related to oxidative stress were identified: H_2O_2 detoxification, SOS response, iron transport and metabolism. Induction of genes related to chemotaxis, flagellum structure and metabolism, cobalamin metabolism and nitrogen regulation system were also observed.
Proteomic analysis clearly showed again a differential production of house keeping, metabolic and stress response proteins between space and ground grown cultures of *Ralstonia metallidurans* CH34. A limited number of metabolic proteins were significantly more concentrated in space grown cells in comparison to ground grown cells, while there is no evidence of reduced protein concentrations due to space flight conditions. Despite the difference in transport and storage conditions and its effects on the cell growth and physiology (see above), the same proteins that were found to change in concentration in space grown cells in the MESSAGE-1 experiment were again detected as altered in concentration in the MESSAGE-2 experiment.
The most significant overproduced metabolic proteins of R. metallidurans CH34 in space flight conditions are probably involved in a metabolic pathway known to be used for acetone catabolism (proteins AcxABC, Ald, ExaC, LpsJ, CaiA). A remarkable observation as only gluconate and no acetone was not provided to bacteria in space flight or ground control experiments. Some other proteins represent possibly a specific stress response to space conditions. Some antioxidant proteins (AhpC, TrxB, DpsA) that protect the cell against peroxides, possibly generated by space radiation, were detected. Also for *R. rubrum* S1H a clear difference in proteome profile between ground and space grown cultures were observed. However, the protein yields obtained after extraction of the samples were too low to enable the identification of the differentially synthesized proteins.
For *R. rubrum* S1H, replica-plating was performed to assess a possible change in *R. rubrum*'s metabolic and resistance profile due to growth under space flight conditions. Under most conditions tested, no differences were observed. One observation, however, needs to be noted. Under light, anaerobic conditions, colonies harvested from space, could only grow in rich medium conditions if tellurate was added to the medium. Tellurate resistance has also been observed in the literature to be induced under stress conditions such as ionising radiation. It might be possible that stresses during or after the incubation in the ISS caused pre-induction of tellurate reduction enzymes and therefore enabled post-flight anaerobic respiration on rich medium containing tellurate.
The assessment of DNA mutagenesis and recombination events induced by space flight conditions did not go as expected. Possibly due to pre-flight cultivation conditions much higher number of mutants appeared in space and ground cultures preventing the good selection of individual mutant colonies in the chosen test set-up. Nevertheless mutants were selected from space and ground cultures post-flight and the nature of the mutation. The current results do not indicate a substantial influence of space flight on the frequency or nature of DNA rearrangements by IS translocation in bacterial genomes but profound bioinformatics and statistical analysis is needed for final conclusions. No mutagenesis experiment was performed on *R. rubrum* S1H.

2.3.1.1.2.3 Conclusions and Recommendations

The MESSAGE-1 and MESSAGE-2 experiments have proven that pre- and post-flight transport and storage conditions as well as in flight incubation conditions (temperature control) are critical parameters for biological space flight experiments that can have a big effect on the results. Adequate flight and ground hardware for

transport and incubation and simultaneous post-flight ground control experiments mimicking the exact culture and storage conditions are very important. Therefore, more sensors and more data concerning for example vibrations, accelerations, temperature and radiation conditions during flight should be made available for all biological research teams and included in all future experiments.
The combined application of different molecular and microbial methods used in the MESSAGE-1 and -2 experiments revealed valuable information to understand better the microbial acclimation and adaptation to space environments. The MESSAGE-1 and -2 experiments have shown that space conditions can significantly change the physiology and metabolism of bacteria. These results of the MESSAGE experiments are of major importance for the development and/or further improvement of bioregenerative systems and systems to detect microorganisms in closed space habitats during future manned long duration missions for space exploration.
More experiments are necessary to further study in detail the microbial response to space flight. There are many other basic aspects of microbial behaviour not yet studied but which could be highly important in closed manned environments in space. In addition new experiments with in-flight controls, i.e. samples that are incubated in space under 1G (on a centrifuge), are essential to elucidate the true effects of microgravity or other space conditions on microbial behaviour. Incubation over a longer period in the ISS, over several generations, would allow to stud long-term effects of space flight on bacterial behaviour. Moreover the study of space flight effects on interactions in bacterial communities or bacterial interaction with other cells of plant, animal or human origin is also of importance.
Only by investigating the basic molecular responses of bacteria to space flight, will accurate data to detect and direct microbial behaviour in space conditions be obtained. These data are indispensable to support future development and application of bacterial life support systems and bacterial detection systems in confined space habitats.

2.3.1.1.2.4 Publications

1. O. Goossens, F. Vanhavere, N. Leys, P. De Boever, D. O'Sullivan, D. Zhou, F. Spurny, E.G. Yukihara, R. Gaza, S.W.S. McKeever, (2006), "Radiation Dosimetry for Microbial Experiments in the International Space Station using Different Track-Etch and Luminescent Detectors", *Radiation Protection Dosimetry, Vol. 120, No. 1–4*, pp. 433–437
2. L. Hendrickx., F. Mastroleo, S. Baatout, C. Paillé, M. Mergeay, (2005), "A global approach to assess stress response of the bioregenerative life support system organism *Rhodospirillum rubrum* ATCC25903 under space-flight related environmental conditions", *SAE Technical Papers, electronic publication 2005-05ICES-358*.
3. N. Leys, R. Wattiez, S. Baatout, P. De Boever, M. Mergeay, (2004), "Gene expression in *Ralstonia metallidurans* CH34 in space", *Habitation - International Journal for Human Support Research, Special issue with Habitation 2004 Conference Abstracts, 4-7 January 2004, Orlando, Florida, USA, Vol. 9, No. 3-4*, pp 117.
4. N. Leys, R. Wattiez, S. Baatout, P. Jansssen, P. De Boever, A. Dams, S. Aendekerke, P. Cornelis, M. Mergeay, (2004), "MESSAGE : Microbial experiments in the space station about gene expression", *International Journal of Astrobiology, Supplement 1, Abstracts from the Astrobiology Science Conference, NASA Ames Research Center, 28 March – 1 April 2004, Moffett Field, California, USA*, pp 68.
5. N. Leys, R. Wattiez, S. Baatout, P. De Boever, A. Dams, M. Mergeay, (2004), "Gene expressions in *Ralstonia metallidurans* CH34 in space flight", *Proceedings of the European Symposium on Environmental Biotechnology, (Ed.) W. Verstraete, (Publ.) A.A. Balkema Publishers, Taylor & Francis Group plc, London, UK*, pp 129-130.
6. N. Leys, L. Hendrickx, P. De Boever, S. Baatout, M. Mergeay, (2004), "Mini review : Space flight effects on bacterial physiology", *Journal of Biological Regulators and Homeostatic Agents, Vol. 18, Issue 3.*

2.3.1.2 Biology: Developmental Biology

2.3.1.2.1 Effects of the gravity altered environment on Drosophilia motility, behaviour and ageing (AGEING)

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2.3.1.2.1.1 Background, Objectives and Procedures

The main scientific objective of the experiment was to study in more detail the mechanisms of the abnormal motility response encountered in space by young flies with consequences on the posterior ageing response of the flies. For this purpose, three different fly strains with different phenotypes were used, in four configurations. The three strains were a long-lived strain, a short-lived strain and a strain showing an abnormal gravitropic response on the ground. Recently hatched flies of the three phenotypes were exposed to the space environment. In addition, a two-week old population of the short-lived strain was also included to confirm the differences between them. During flight, the only experimental activity planned was the video recording of the in flight motility in the different experimental containers. This was complemented by an extensive series of post-flight analyses involving behavioural assays (gravitropic responses, mating activity of the males, optokinetic responses, gene expression profiles and neuropeptide patterns of defined neurons).
The experiment was complemented by appropriate ground controls involving space simulation exposures of equivalent groups of flies.

Several hours before docking, the Aquarius B Incubator in the Russian segment of the ISS was switched on so that a temperature of 22ºC was reached and stabilised when the biology experiments arrived at the Station. Upon arrival the Biology Transport Container was removed from the Soyuz Capsule. The AGEING biology container was opened and removed and inserted into the Aquarius incubator (22°C ±3ºC). On day 2 in the ISS, the first video-recording session was performed. The AGEING containers (Figure 2-10) were removed from the Aquarius B Incubator and video-recorded four at a time, for 5-15 minutes. Since the experiment used eight containers, and the holder could take four at a time, the activity was split in two steps. After completing each video-recording step, the containers were transferred back to the Aquarius B Incubator. The camera was mounted and the tape inserted.
On day 4 in the ISS the second video-recording session was performed using the same procedure as on day 2. On day 6 in the ISS, the third and final video-recording session was performed using the same procedure as on day 2 and 4. At the end of the mission, the containers were removed from the Aquarius B Incubator and introduced back into the Biology Transport Container, together with the two tapes and the temperature data logger.
The final operation was the transfer of the Transport Container into the Soyuz Capsule. Early retrieval was requested for this experiment.

Figure 2-10: Internal part of AGEING container with flies

2.3.1.2.1.2 Results

Not available.

2.3.1.2.1.3 Conclusions and recommendations

Not available.

2.3.1.2.1.4 Publications

Not available.

2.3.1.3 Physiology: Integrative gravitational physiology

2.3.1.3.1 Directed attention brain potentials in virtual 3-D space in weightlessness (NeuroCOG)

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2.3.1.3.1.1 Background, Objectives and Procedures

The NeuroCOG experiment was designed to further investigate modifications in the perception of whole-body motion in space found during the French Soyuz Mission, "Andromede", in October 2001. The NeuroCOG experiment went a step further in understanding the neural mechanisms underlying the perceptual processes by combining the psychophysical experiments with measurements of visually-evoked EEG potentials.

The human being in his natural environment moves, because of the constraints of gravity, over a relatively flat two-dimensional surface. During Earth-bound navigation, only yaw rotations are typically used when moving from one place to another. Even when moving through three-dimensional structures, human beings tend to remain upright with respect to gravity. In weightlessness, astronauts can translate and rotate in any direction, thus their trajectory is no longer ascribed to two-dimensional surfaces. In contrast with Earth-bound navigation, astronauts can freely use pitch and roll rotations when moving through three-dimensional space. The semi-circular canals measure relative rotations around all three axes (roll, pitch and yaw). This provides relative information about the amplitude of a rotation, but does not provide absolute information about orientation. The otoliths and other graviceptor cues (tactile sensors, proprioception, etc.) can potentially indicate the absolute orientation of the head and body with respect to the vertical axis. Neural processes that allow us to perceive, interact and navigate within this world may thus be specialised for the internal representation of spatial relationships with respect to gravity.
The novel conditions of microgravity might therefore place an increased load on the cognitive capacity of the human brain because sensory signals must be processed and interpreted in a new context. By placing electrodes on the scalp of a human subject one can get a glimpse at the electrical activity underlying perceptual processes in the brain. Through the analysis of the variations in electrical potential between different locations on the scalp, one can make inferences about various neural processes such as the sensitivity to sensory information, the attention state of the system and the decision making process.
This project studies how the brain functions with respect to gravity through the use of these techniques known as electroencephalography or EEG. In this experiment the role of gravity in the perception of self-motion is tested. In a series of psychophysical tests, a comparison is made on how human subjects interpret visual-flow information

both on the ground and in the weightless conditions of orbital flight. Also, evoked potentials through surface electrodes applied to the scalp are measured in order to determine the spatial and temporal components of information processing in the brain in the absence of gravity. Through these experiments observations were made on how the CNS (Central Nervous System) adapts from its habitual environment in which gravity plays an ever-present and dominant role, to a novel environment in which the movements of our bodies no longer adhere to the constraints imposed by gravity.

The hypothesis is that gravity should influence the perception of pitch but not yaw turns. Performing perception tasks in 0g should evoke different cognitive responses and should activate different cortical circuits, depending on whether the information to be interpreted by the subject involved turns around a pitch or yaw axis.

Subjects performed a set of 2 psychophysical tasks with simultaneous recording of EEG activity. For each subject, the performance of these tasks was compared to a set of pre-flight, in-flight and post-flight procedures to test for an effect of weightlessness on the visual perception of orientation and movement and on the ability to navigate in three dimensions. Backup crewmembers were asked to perform all pre-flight training and baseline data collection (BDC) tests and were asked to work in parallel with the orbital crew during and after the flight to provide a matched control group for comparison.
Subjects take up the position and postural support depending on the gravitational conditions (ground or in-flight) and on the instructions for a particular protocol:

1. *Ground Seated*: The subject sits upright in a chair, with the elbows resting on adjustable-height elbow supports of the ground support stand. The ground support stand is adjusted to position the mask/tunnel/laptop at the level of the eyes for viewing. The height of the elbow pads is adjusted to allow the subject to comfortably grasp the grips on the laptop support.
2. *In-flight Restrained*: The subject sits in front of the laptop, which is attached to a mechanical support. Waist and foot straps are used to hold the subject securely in a seated posture.
3. *In-flight Free floating*: The subject adopts a free-floating posture and has no rigid contact with the Station structure during the performance of the experiment in this mode. A second cosmonaut assists the subject to stabilise his/her posture at the beginning of this phase of the experiment.

In all cases, the subject places his/her face into the facemask and attaches an elastic band behind the head to hold it in place. By manipulating the buttons and trackball, the subject launches the experiment program on the laptop, identifying him/herself to the program and performs a set of experimental trials consisting of the following:

- FO1: Virtual Turns – The subject is situated in a visually-presented 3-D virtual tunnel. On the press of a button, the subject appears to either move through a tunnel at constant speed, passing through a single bend between two linear segments or the subject appears to undergo a rotation in place (no apparent translation). At the end of the trial, the subject indicates the extent of the turn (i.e. how many degrees) in one of two ways:
    1. The subject observes a bird's eye view of a planar workspace with two cylindrical tunnels connected by a variable angle. By manipulating the trackball, the subject adjusts the magnitude of the turn to reconstruct a planar representation of the virtual tunnel just experienced.
    2. The subject sees a pictogram indicating his/her starting orientation in the plane. By manipulating the trackball, the subject changes the orientation of the pictogram to indicate the amount of rotation that is perceived.
- EEG Recordings – EEG signals from 14 locations on the scalp are recorded during the above trials. The subject performs a total of 48 trials for either stimulus type, for a total of 96 trials per session. Trials are broken into blocks of 12 trials each, with pauses programmed between blocks. At a nominal rate of 4-5 trials per minute (including pauses), one complete execution of this protocol (turning in-place or passage through the tunnels) is performed in 20-25 minutes. EEG is also recorded under four control conditions:
    1. The subject relaxes and does nothing, first with his/her eyes closed, then while looking at a neutral screen.
    2. An alternating checkerboard is presented to the subject on the screen, with the colours switching between black and white every 3 seconds.
    3. The subject follows the movement of a luminous spot as it makes a sinusoidal movement across the screen.
    4. Subjects blink their eyes in synchrony with an audible metronome. Control recordings last no more than 5 minutes.

2.3.1.3.1.2 Results

After subjects emerged from the end of the tunnel, they were asked to report the perceived turn angle by adjusting a visual indicator with a trackball. Figure 2-11 shows the difference between left and right turns (left–right) and between upward and downward turns (downward–upward) as a measure of this asymmetry. On Earth, yaw turns led to equal, symmetrical errors in the estimation of the perceived angle change, but the estimation of pitch turns was greater for forward (nose-down) versus backward (nose-up) turns. The interest of this experiment lies in this asymmetry. One can observe a clear reduction in the asymmetry of vertical turns in 0g. In summary, it appears that the microgravity conditions of orbit reduce the asymmetry of vertical turn estimation, but only in the free-floating condition. The NeuroCOG experiment revealed interesting EEG correlations of these effects observed via psychophysics. Alpha rhythms were analysed in response to a standard alternating checkerboard pattern (visual evoked potential, VEP) and in response to the initial presentation of the virtual 3D tunnel (event related potentials, ERP). It was demonstrated for the first time that the VEP responses are conserved in the absence of gravity and that the phase locking of alpha rhythms is preserved in the ISS environment. In contrast, the ERP evoked by the presentation of the tunnel was dramatically perturbed in the ISS. Unspecific factors such as a noisy environment in the ISS, anxiety, stress, muscular artefacts and basic physiological factors (brain and body blood circulation differences) seem to be unlikely culprits because the classical VEP in response to the reversing checkerboard pattern is maintained. This latter phenomenon occurs through the conservation of the phase locking mechanism of the VEP in the alpha band frequency, as seen on the ground. These results (see Figure 2-12) can be interpreted in light of the specific informational content of the different visual stimuli (checkerboard vs. tunnel) that the associated task demands. The major difference between the classical checkerboard testing and the virtual tunnel task is that in the former situation the subject was mainly passive (only looking at the computer screen) while in the latter situation the subject was involved in a 3D spatial perception task. This task directly contained directional information related to the gravitational frame of reference, which may play the role of a top-down control. In the presence of gravity, this neural context implicitly contributed to the evoked response. Thus a change of perceptual context or a basic interference in the dynamics of the neural networks could be expected, resulting in the different patterns in EEG measurements between the 2 tasks.

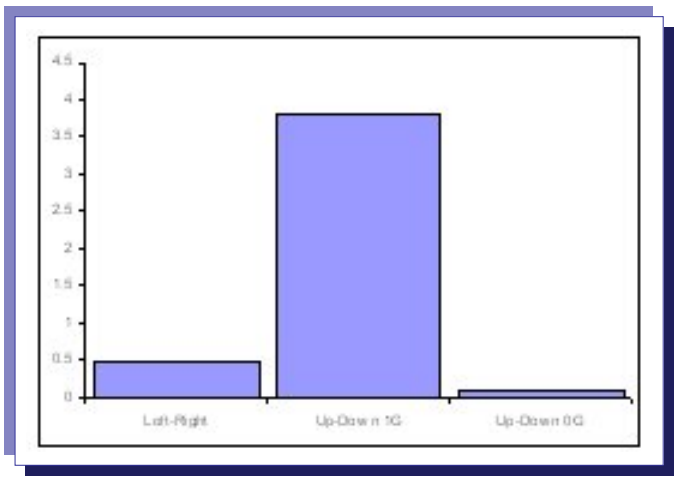

Figure 2-11: Asymmetry in the estimation of turn angles for virtual rotations around horizontal and vertical axes

In the NeuroCOG experiment before the navigation task, the arrest reaction of the alpha rhythm was used (Berger, 1929) because it is a highly stable reaction, which occurs over a large part of the brain and provides two distinct physiological states induced by opening or closing the eyes. The head figurines of Figure 2-13 illustrate the difference in the power gain of 10 Hz activity between the recordings performed in the ISS and on Earth (with data recorded before and after flight pooled together) for cosmonauts (A) and for control subjects (B). Statistical analysis revealed that the gain values recorded in parieto-occipital (O1, O2, Pz, P3, P4) and central (C3, C4, Cz) loci were significantly increased in weightlessness. The three latter electrodes are situated over the sensorimotor cortex, which is the site of the mu rhythm. In contrast, the 10 Hz gain value of the frontal recordings (Fz, F3, F4, F7, F8) remained unchanged in the absence of gravity. The same analysis performed in the control subjects showed a great stability of the gain value throughout the same period of time in all recorded channels. The

findings demonstrate that the power of the spontaneous EEG alpha rhythm recorded in the parieto-occipital regions and in the sensorimotor areas (mu rhythm) are increased and that the spectral perturbations of these rhythmic activities produced by eye-opening/closure state transition, increase in the absence of gravity. This demonstrates the influence of the absence of gravity on alpha oscillation, which is likely to be linked to the gating of sensory input. Alpha and mu rhythms may also participate in memory and cognitive processing. In this context, the finding of enhanced alpha and mu rhythm in weightlessness supports their physiological implication in the gain-field mechanism allowing the adaptation of the neural representation of space.

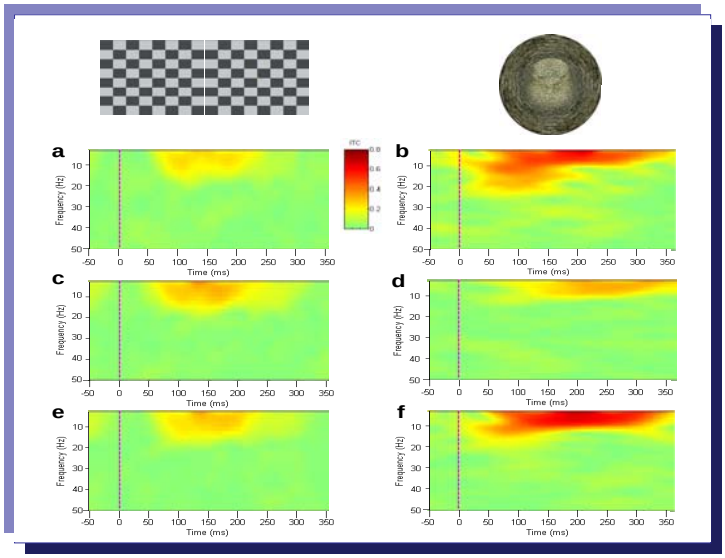

Figure 2-12: Inter-trials coherence of theta and alpha rhythms in response to a standard checkerboard pattern (a, c, e) and to the presentation of a curved tunnel (b, d ,f) on the ground before flight (a, b) in flight (c,d) and on the ground after flight (e, f)

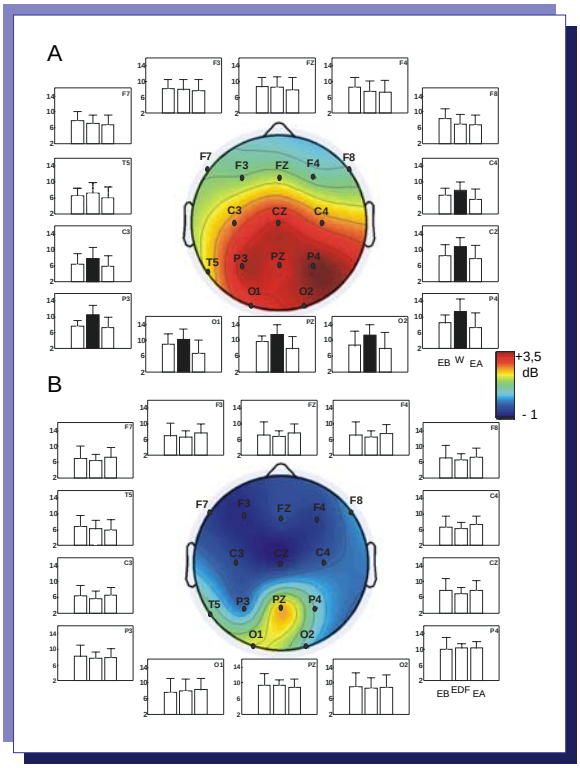

Figure 2-13: Difference in the power gain of 10 Hz activity between the recordings performed in the ISS and on Earth

Motion-onset related visual evoked potentials (M-VEPs) were recorded at a latency of ~200 ms (N200) when the first in depth motion appeared during the virtual navigation. The N200 was supported by a very significant phase locking in the theta range oscillation. It was shown for the first time that this N200 and the related phase locking in theta oscillation are suppressed during the first days in weightlessness and that this effect is reinforced in free-floating condition. Interestingly, this M-VEP reappeared with the time passed in weightlessness and will be carefully followed in the long-term ISS missions.

2.3.1.3.1.3 Conclusions and Recommendations

Three main conclusions can be made from the results obtained:

1. Weightlessness specifically affects event related potential related to the presentation of a virtual 3-D navigation tunnel.
2. Weightlessness increases alpha rhythm gain during transition between eyes-closed and eyes-open states.
3. Moving in virtual navigation induced midfrontal N200 event related potentials supported by a transient theta ringing altered in weightlessness.

In any given EEG recording session a complete loss of signal (flat line) was sometimes observed on one or more of the 14 EEG channels. This loss of signal is often accompanied by a zero impedance level during the impedance check prior to the start of recording. This may be due to changing characteristics of the conductive cream with time, differences in environmental conditions between ground and flight (humidity, temperature, etc.) or due to the application of a greater quantity of cream during in-flight sessions than on the ground. Post-flight debriefing with the cosmonaut suggested that the latter may have been the case.
Future experiments using EEG should provide real-time or quasi-real-time monitoring of EEG signals on the ground. Downlink should be timely enough to allow for the correction of problems during the course of a data collection session, or at least soon enough to allow for the repetition of a session in the case of data loss.
The assurance of adequate training time should be a critical factor in the planning of future experiments.

2.3.1.3.1.4 Publications

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2. G. Cheron, A. Leroy, C. De Saedeleer, A. Bengoetxea, M. Lipshits, A. Cebolla, L. Servais, B. Dan, A. Berthoz, J. McIntyre, (2006), "Effect of gravity on human spontaneous 10-Hz electroencephalographic oscillations during the arrest reaction", *Brain Research, Vol. 1121* , pp. 104-116
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4. G. Cheron, A. Leroy, C. De Saedeleer, A. Bengoetxea, M. Lipshits, A. Cebolla, A. Berthoz, J. McIntyre, (2005), "Alteration of the visual evoked potentials related to the presentation of a virtual 3D tunnel in weightlessness", *Life in Space for life on Earth (Cologne2005),* pp. 213-214
5. J. McIntyre, G. Cheron, C. De Saedeleer, A. Bengoetxea, M. Lipshits, A. Cebolla, A. Berthoz, M. Vidal, D. Chaput, E. Lorigny, (2005), "Effects of gravity on perceiving the angle of turns during virtual movement", *Life in Space for life on Earth (Cologne2005),* pp. 236
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2.3.1.3.2 Cardiovascular adaptation to weightlessness (CARDIOCOG-1)

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2.3.1.3.2.1 Background, Objectives and Procedures

Orthostatic 'intolerance' (OI), an important physiological consequence of human space flight, is primarily characterised by a fall in stroke volume in the upright position after landing. The underlying pathophysiological mechanisms of OI have been investigated extensively, but no single satisfactory explanation has been proposed yet.
The aim of this study was to asses the relative contribution of (1) autonomic baroreceptor responses and (2) circulatory adjustments in different topographic vascular beds to the mechanism of OI.

To achieve these goals the following experimental design was proposed:

1. a standard computerised (HICOPS) protocol combining supine and standing provocative tests: head flexion, arrhythmic stress, fixed respiration (pre-, in-, and post-flight);
2. a tilt test protocol that provides insight into circulatory control during orthostatic (pre- and post flight);
3. a 24h ECG Holter-protocol (pre- and post flight) to investigate circadian variations of cardiovascular autonomic control;
4. cerebral and lower limb flow measurements (echo-Doppler) that provided data on circulatory regulation in different vascular beds (cerebrovascular, femoral, splanchnic), (pre- and post-flight).

The ECG, blood pressure (non-invasive Portapres), echo/Doppler and respiration (abdominal sensor) were continuously recorded and analysed off-line using linear and non-linear techniques of heart rate variability (HRV), blood pressure variability (BPV) and baroreflex sensitivity (BRS). Changes in hemodynamic parameters (stroke volume, cardiac output and total peripheral resistance) were estimated by modelling flow from finger arterial pressure (Modelflow).

The hypotheses underlying this study are that:

1. the observed perturbations in autonomic cardiovascular (baroreflex) control may be more severe after long duration space flight (6 months) leading to more pronounced problems of orthostatic intolerance compared to the alterations observed after short duration (10 days) space flight;
2. the duration of return to normal values after long space flights might persist at least 25 days after return;
3. the maximal flow velocity will be altered at the lower limb arterial level whereas it will not be affected at the cerebral level;
4. the flow supplying the splanchnic area may not reduce in response to fluid shift towards the legs (orthostatic test) which could contribute to make the cardiac output redistribution towards the brain less efficient.

The different tests and measurements that were applied in the protocols allowed to investigate the different functions of the autonomic nervous system and to study hemodynamics. The research objectives were as follows:

1. How is the autonomic control of both heart rate and blood pressure affected during these long-term missions? How is the baroreflex system affected during 6 months in space?
2. What is the time frame in which these changes take place? Do the changes continue after 2 weeks in space, or is an equilibrium reached?
3. Is orthostatic intolerance more severe after long duration than after short duration spaceflight?
4. What is the relative contribution of baroreflex control of heart rate and total peripheral resistance in the recovery after spaceflight and the decrease in orthostatic tolerance?
5. How does blood flow in cerebral/vascular beds influence the observed decrease in cerebral/femoral flow ratio (calf vein, portal vein) contributing to a reduction in brain blood supply leading to OI?

CARDIOCOG-1 was conducted as part of the experimental package of the ESA supported Spanish Soyuz Mission, "Cervantes" (ISS 7S mission), which took place in October 2003 during increment 8. CARDIOCOG-1 was continued during increments 9 and 10.
The experimental protocols were performed by 5 cosmonauts before, during and after a 10-day mission and by 2 cosmonauts during a 6-month mission, and contributed in determining the differences in autonomic cardiovascular modulation between long and short term spaceflight.

All in-flight protocol measurements were non-invasive. The cosmonaut was guided through the experiment with a software program developed by a member of the research group and this program was used during all previous missions using the CARDIOCOG protocol. A minimum of 4 repetitions were executed in-flight, with a total in-flight experiment duration of 120 minutes per subject.
The pre- and post-flight baseline data collections (BDCs) of the CARDIOCOG protocol were performed in 3 postures (supine, sitting and standing): The cosmonaut was guided through the experiment with a software program developed by a member of the research group. The timeframe for the pre-flight session was Launch-50 days. In order to execute a comparison with the findings of previous missions it was important to reproduce these results at more or less the same days. Especially the first days were critical and the long-term follow-up. The timeframe for the post-flight measurements was R+1, 7, 10, 20, 30 and R+40. Because of limitations in cosmonaut time in the first days after return, a shortened version of the CARDIOCOG protocol was proposed at R+1 (duration <1 hour).

The variables measured during the experimental protocols were the following:

- Respiration (an important modulator of HRV) was monitored continuously (pressure sensor on abdomen).
- *ECG* electrodes were applied to the chest wall.
- *Continuous blood pressure* was determined (Portapres) with a non-invasive pulse method at the finger and converted to brachial blood pressure
- *Cerebral flow and vascular resistance, Femoral flow and vascular resistance* Flow distribution between these areas and total regional sympathetic power at cerebral and lower limb level. Main arteries were

investigated by Doppler ultrasound using sensors fixed on the skin by strap and bandeau. For the cerebral flow the Doppler probe was placed at the upper level of the zygomatic arch facing the middle cerebral artery, the appropriate orientation was found with help of a rotula. The rotula was mounted on a head helmet in order to maintain a stable orientation. For the femoral artery the probe was placed in front of the superficial femoral artery, at the upper part of the thigh, and was fixed using 2 thigh and abdominal belts. This Cardiolab Doppler system (around 5 kg) allowed continuous and simultaneous recording of the flow velocities in 3 vessels. A similar Doppler system was used several times in-flight on cosmonauts while performing LBNP test onboard MIR then during pre and post flight Stand test. The system was also used on head down tilt (HDT) subjects.

- *Venous echography, Calf and Portal veins:* (Portal vein flow and diameter, Tibial and gastrocnemian vein section, Tissue (calf muscle) liquid content) These 2 central and peripheral veins selected were investigated by echography, the probe being fixed on the calf skin at the posterior face of the calf, the probe being handled by a sonographer for the Portal vein. The echographic image of calf vein was recorded in real time (at rest or during stand tests) while the Portal vein image was recorded time by time.

2.3.1.3.2.2 Results

Not available.

2.3.1.3.2.3 Conclusions and Recommendations

The real applications will have to be investigated further in the future, but some advantages with the data for clinical studies in syncope patients are expected. These patients often faint (some kind of orthostatic intolerance) and the cosmonaut data can help provide insights in the cardiovascular control mechanisms and how it responds to changes in adaptability to orthostatic stress.
Pre- and post flight schedules could be communicated earlier to the investigators (even if these concern preliminary schedules). Especially for teams that involve international collaborations this can be very helpful in planning and coordinating the experiments.

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4. F. Beckers, B. Verheyden, A.E. Aubert, (In Press 2006), "Space Physiology", *Wiley Encyclopedia of Biomedical Engineering*
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2.3.1.3.3 Sympathoadrenal activity in humans during spaceflight (SYMPATHO-1)

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2.3.1.3.3.1 Background, Objectives and Procedures

It was previously shown that sympathoadrenal activity contrary to expectation was increased during spaceflight as compared to ground-based observations in the supine position. Plasma norepinephrine (NE) concentrations were increased in four astronauts studied on the 5th and 6th day of the D2-mission (J Appl Physiol 1995; 78: 2253-2259). Furthermore, urinary excretion rates of both NE and epinephrine (E) obtained from two astronauts during a MIR-mission were above supine levels obtained in ground-based experiments. No correlation was obtained between urinary excretion rate of NE and E and the length of the mission between day 5 and 164 (Lancet 2000; 356: 1577-1578).
Therefore the following hypothesis was proposed: Sympathoadrenal activity is low during the first 24 hours as suggested by simulation studies of microgravity, but the activity increases subsequently due to a pronounced decrease in intravascular volume.
The sympathetic system is that part of the nervous system that accelerates the heart rate, constricts blood vessels, and raises blood pressure. To test this hypothesis, measurements of mean 24-hours plasma NE and E concentrations as evaluated by quantification of thrombocyte NE and E concentrations were proposed. These measurements are not dependent on renal function and included NE derived from the gastrointestinal tract. The ratio between plasma NE and thrombocyte NE and between plasma E and thrombocyte E is constant and independent of the actual plasma level. The half-time of thrombocyte NE is approximately 2 days. Blood samples were therefore obtained before the mission and early post flight. In addition thrombocyte NE and E in blood samples obtained in ground based experiments were measured before and during head-down bed rest. Plasma and thrombocyte NE and E were analysed by a sensitive and precise radioenzymatic assay.

From the research it was expected to find increased thrombocyte NE and E concentrations early post flight indicating increased sympathoadrenal activity during spaceflight. Furthermore, it was also expected to find a low 24-hours sympathoadrenal activity in the head-down bed rest study.
Previous studies and the proposed study are of importance to the space programme, because the present findings indicate that the head-down tilted procedure cannot be applied to simulate microgravity as observed in space. It is therefore important to develop another more reliable model for simulation of microgravity. Furthermore, the elevated plasma NE and E concentrations observed in space may have an important secondary effect on cellular function in the immune system and in the endocrine system.
All study protocols were reviewed and approved by Ethics Committees at the ESA Medical Board and were in compliance with the declaration of Helsinki II.

SYMPATHO-1 was conducted as part of the experimental package of the ESA supported Spanish Soyuz Mission, "Cervantes" (ISS 7S mission), which took place in October 2003 during increment 8. SYMPATHO-1 was continued during increments 9, 10 and 11.

The Head Down Bed Rest (HDBR) study.
Nine normal subjects participated in all 4 study phases (see Table 2-4).

Table 2-4: Study design and phases

ADAPTATION PERIOD (9 DAYS)	INTERVENTION PERIOD (14 DAYS)
Normocaloric diet, ambulatory	Phase 1: Normocaloric diet, ambulatory
	Phase 2: Normocaloric diet, 6° head-down tilt
	Phase 3: Hypocaloric diet, ambulatory
	Phase 4: Hypocaloric diet, 6° head-down tilt

One subject participated only in phase 1 and another subject participated in the following three study periods. The mean age was 23.8 years (range 21 to 29 years). The mean body mass index was 23.0 kg/m^2 (range 19.2 to 27.8 kg/m^2). All subjects were healthy and had a normal heart rate and blood pressure.
Study phase 1 and 2 started with an adaptation period of 9 days (-9 to -1) where the subjects were ambulatory. This was followed by an intervention period of 14 days (+1 to +14), where the subjects at random were either ambulatory or subjected to -6° HDBR and vice versa. Study phase 3 and 4 was performed in the same way except that all subjects were on a hypocaloric diet during the intervention period. Phase 2 and 4 were the HDBR study and phase 1 and 3 the ambulatory study.
The daily normocaloric diet consisted of protein (1 g per kg body weight per day), fat (30% of the energy, the fatty acid composition was saturated and polyunsaturated fatty acids) and carbohydrate (remaining calories). In addition the subjects received 50 ml of water per kg body weight, 2.5 mmol sodium/kg, 1000 mg Calcium and 400 IU vitamin D per day. The hypocaloric diet had an energy intake of 75% of the respective normocaloric ambulatory diet.
All subjects received the same amounts of water, protein, sodium, calcium and vitamin D. Intake of alcohol and caffeine was not allowed. All other nutrients without experiment-specific requirement matched dietary recommended intake levels of the German Nutrition Society. In the adaptation period in the 4 phases all subjects received the normocaloric diet of identical nutrient composition. Total energy expenditure was calculated as basal metabolic rate multiplied by the physical workload plus the calculated thermic effect of feeding.
The test subjects stayed in the laboratory at all times also during the ambulatory study phases. Subjects were not allowed to do any exercise on a voluntary basis. However, in phase 1 and 3 subjects followed an exercise protocol, which was two times 15 minutes of bicycle ergometry (about 125W).
Blood samples for plasma and platelet catecholamines were collected from an antecubital vein. The samples were always collected in the morning at 7 a.m. with subjects in the supine position. The blood samples were immediately brought to the laboratory and prepared for analysis. Blood samples were obtained on day -4 and day -2 in the adaptation period and again on day +5, +9 and +14 during the intervention period. No samples for catecholamine analysis were obtained in the recovery period.
For practical reasons, the preparation of blood samples differed in the HDBR study compared to the microgravity study. For this reason no comparison was made of absolute values between the two groups. Relative changes observed in relation to the corresponding basal values in the adaptation period and pre-flight were compared.

Spaceflight study
Blood samples for platelet measurements were collected from an antecubital vein in 5 male cosmonauts. The mean age was 41 years (range 37 to 45 years). The cosmonauts participated in 3 Soyuz missions to the International Space Station. Samples were collected approximately 14 days before launch, after 11 to 12 days in flight, within 12 hours upon landing and finally at least 14 days thereafter.
Samples for platelet norepinephrine and epinephrine measurements should preferably have been obtained in-flight, but this was not possible because no centrifuge with an adjustable speed was available on the International Space Station. Due to the long half life of platelet norepinephrine and epinephrine (see below) a sample taken after 11 to 12 days in flight and within 12 hour after landing would still reflect the microgravity state. The half life of platelet norepinephrine was tested in 5 normal subjects during the first 4 days of another HDBR study.
The mean half life for platelet norepinephrine was 54 ± 12.5 hours. There was a tendency for an inverse relationship between the half life and the basal platelet norepinephrine values.
The platelets could not be counted at the sampling site and therefore the preparation of the platelets had to be modified. After the initial centrifugation at 350 G, samples of 0.5 ml were added to eppendorf tubes and centrifuged, decanted and frozen at -20°C. In addition at least two times 0.3 ml plasma samples were obtained and added to eppendorf tubes. These samples were not centrifuged but frozen and later applied for counting the number of platelets. A preliminary study indicated that the number of platelets remained the same before and after freezing. The mean platelet level in the cosmonauts pre-flight and within 12 hours after landing averaged 243 ± 20 and 261 ± 56 x 10^9/l. These values were not significantly different and well within normal range (140-340 x 10^9 platelets/l). Plasma and platelet norepinephrine and epinephrine concentrations were quantified by a sensitive and precise radioenzymatic assay.

2.3.1.3.3.2 Results

Platelet norepinephrine decreased significantly during HDBR. The tendency for platelet norepinephrine to decrease during the normocaloric ambulatory study was not significant. The hypocaloric diet had no effect on platelet norepinephrine levels, which decreased during the HDBR but remained unchanged during the ambulatory study period. The mean platelet norepinephrine level in the four experiments in the adaptation period before the intervention averaged 42.9 ± 9.8 (mean ± Standard Error (SE); *phase 1*), 41.2 ± 7.4 (*phase 2*), 34.4 ± 8.3 (*phase 3*), and 32.9 ± 8.3 (*phase 4*) pg/10^8 platelets. The values obtained in the adaptation period in phases 1 and 2 tended to be higher than in phases 3 and 4 .
Platelet norepinephrine levels varied between individual subjects, but values in the same subject in the two samples obtained in the four adaptation periods were correlated. There was also a strong positive correlation between platelet norepinephrine values in the adaptation period and during the intervention, indicating that the relative decrease in platelet norepinephrine was approximately the same in all subjects. The corresponding values for platelet epinephrine in the adaptation period were 2.7 ± 0.7 (*phase 1*), 2.9 ± 0.9, 2.3 ± 0.8, and 2.6 ± 0.5 pg/10^8 platelets (not significant). Platelet epinephrine did not change significantly during HDBR and was not influenced by the hypocaloric diet.

Table 2-5: Plasma norepinephrine in 10 normal subjects during the adaptation and intervention periods

PHASE	DAY -4	DAY -2	DAY 5	DAY 9	DAY 14	P-VALUE
1	0.18 ± 0.04	0.11 ± 0.02	0.12 ± 0.04	0.11 ± 0.03	0.15 ± 0.04	Not significant
2	0.24 ± 0.06	0.15 ± 0.04	0.09 ± 0.01	0.09 ± 0.01	0.11 ± 0.01	0.01
3	0.16 ± 0.03	0.09 ± 0.01	0.07 ± 0.01	0.11 ± 0.03	0.22 ± 0.05	0.002
4	0.21 ± 0.04	0.09 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.11 ± 0.02	0.001

Table 2-5 shows plasma norepinephrine during the four phases. In the HDBR with normocaloric diet plasma norepinephrine decreased significantly, but the decrease occurred already between the first and second sample in the adaptation period. A similar response was seen in the adaptation period of phase 4. During phase 3 (hypocaloric and ambulatory), plasma norepinephrine increased significantly at the end of the intervention period. Thus there was no change in plasma norepinephrine that could be related to HDBR. Plasma epinephrine values were low, with mean values ranging from 0.00 to 0.02 ng/ml. No significant differences were observed.

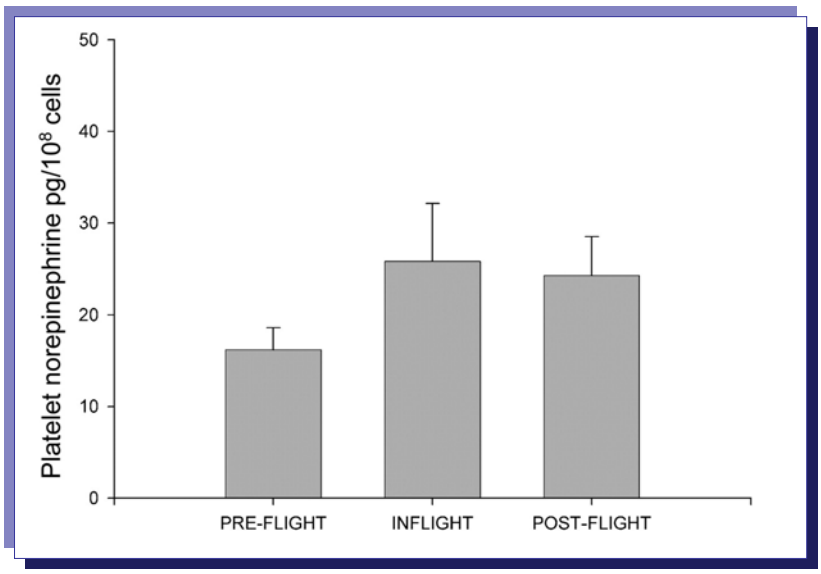

Figure 2-14: Mean platelet norepinephrine values (+/- SE) in 5 cosmonauts (one value was missing postflight)

Figure 2-14 shows platelet norepinephrine values in the cosmonauts. Pre-flight values were within normal range but in the lower end. Epinephrine values averaged pre-flight 1.5 ± 0.3, in-flight 3.8 ± 1.1, and post flight 2.1 ± 0.2 pg/10^8 platelets. Platelets from subjects participating in the HDBR study and from the cosmonauts were processed and stored in different ways, and a comparison of the absolute values may not be relevant. The relative changes may, however, be compared. Platelet norepinephrine during microgravity and during HDBR expressed in percentage of basal values (pre-flight or pre-HDBR values, respectively) were significantly different (152.6 ± 28 vs. 59.8 ± 5.7%) for a difference between in-flight and HDBR (Figure 2-15).

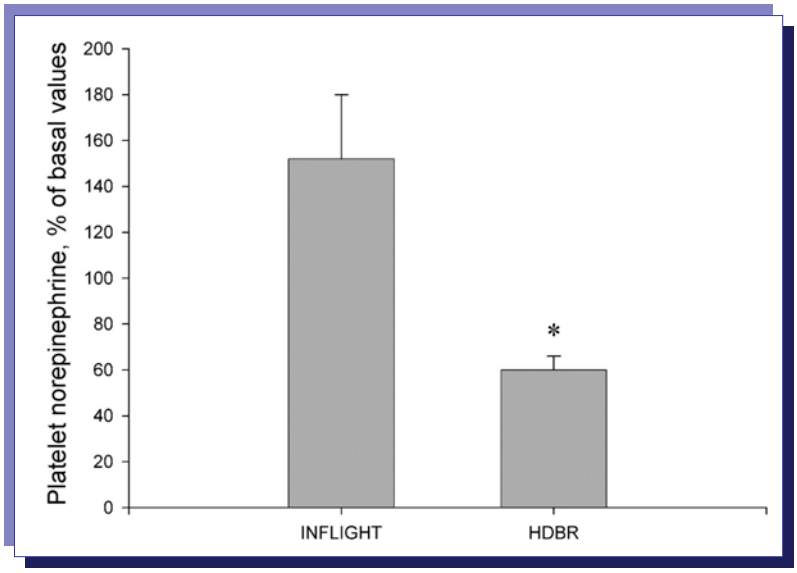

Figure 2-15: Platelet norepinephrine during microgravity and during HDBR expressed in percentage of basal values

Comparison of in-flight values with values from the phase 4 study was also significant (152.6 ± 28 vs. 57 ± 6.6%). Comparing platelet epinephrine in the same way as norepinephrine indicated that platelet epinephrine was significantly different during microgravity compared with the HDBR experiment [293 ± 85 vs. 90 ± 12% (*phase 2*) and 89 ± 18% (*phase 4*)]. Thus there was a marked and highly significant difference in platelet norepinephrine and epinephrine responses during microgravity compared with HDBR. The lack of a decrease in platelet norepinephrine in cosmonauts compared with participants in the HDBR study cannot be explained by the relatively lower pre-flight values. In the phase 4 study, four subjects had mean values in the adaptation period below 20 pg/10^8 platelets (range from 5 to 17.5), and all values decreased during HDBR.

2.3.1.3.3.3 Conclusions and Recommendations

Several studies have now demonstrated, contrary to expectations, that sympathetic nervous activity is not decreased during microgravity, and it is most likely increased compared with ground-based values. In 1995 it was reported that plasma norepinephrine values were elevated in-flight and above values observed in the seated position in ground-based experiments. Ertl et al. concluded that baseline sympathetic neural outflow was increased moderately in-flight. Furthermore, in the same study it was demonstrated that the norepinephrine spillover rate was significantly increased in space. In this study, the steady-state concentration of the norepinephrine tracer was measured in venous blood and not in arterial blood, and the calculated clearance values are therefore too high and to some extent dependent on variations in the local uptake of the tracer in the forearm tissue. Results of the present study are in accordance with previous study in which it was shown that plasma norepinephrine concentrations were increased during microgravity. Thus results from all three studies, which applied different techniques to study sympathetic nervous activity, support the concept that sympathetic activity is moderately increased during microgravity. The platelet measurements showed high epinephrine values during microgravity that were not observed in the previous study. The reason may be that in the first study samples were obtained from a forearm vein and epinephrine in arterial blood is extracted by forearm tissues. Platelets circulate through all parts of the body and take up catecholamines from plasma. Platelet epinephrine may therefore be a more reliable index of epinephrine release in the body than epinephrine in forearm venous blood. The exact interrelationship during microgravity between the initial increase and gradual decrease thereafter in cardiac output and plasma volume and the increment in sympathetic nervous activity during spaceflight remains to be elucidated. Furthermore, the difference in the norepinephrine response to HDBR and microgravity should also be explained. Most likely the decrease in plasma volume in-flight plays a major role for the increase in sympathetic nervous activity. There it does not appear to be a pronounced early increase in urine output during weightlessness, but there may be a relative increase compared with the intake of fluid, because fluid and food intake decreases. The reduction in plasma volume during HDBR has little influence on basal sympathetic nervous activity as long as the subjects are supine. After prolonged bed rest the subjects have a tendency to develop orthostatic hypotension, which largely can be corrected for by fluid intake. The relationship between cardiac output and sympathetic nervous activity during spaceflight is more difficult to explain. The dilatation of the cardiopulmonary area during spaceflight should inhibit sympathetic nervous activity, but at the same time the distended central vasculature would induce a decrease in vascular compliance. This is probably also what occurs during the early period of spaceflight as observed during parabolic flights. During the subsequent decrease in cardiac output and stroke volume, arterial pulsation will decrease. This decrease in pulsation combined with a decrease in compliance of the central vascular wall may activate sympathetic nervous activity.

The possibility that changes in sympathetic activity during microgravity are due to a decrease in the sensitivity to catecholamines cannot be excluded, but this suggestion can hardly explain the difference between bed rest and microgravity.

Furthermore, the sympathetic response during spaceflight is also unlikely to be an arousal reaction due to mental stress, because neither blood pressure nor heart rate increased in-flight. In conclusion, a relative high sympathoadrenal activity compared with pre-flight values seems to be an integrated part of the regulatory response to microgravity. Furthermore, HDBR cannot be applied to simulate changes in sympathoadrenal activity in humans during microgravity.

2.3.1.3.3.4 Publications

1. N. J. Christensen, M. Heer, K. Ivanova, P. Norsk, (2005), "Sympathetic nervous activity decreases during head-down bed rest but not during microgravity", *J Appl Physiol, 99*, pp. 1552-1557

2.3.2 Physical Sciences

2.3.2.1 Material Sciences: New materials, products and processes

2.3.2.1.1 Counterdiffusion protein crystallisation in microgravity and its observation with the Protein Microscope for the ISS (PromISS-2)

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2.3.2.1.1.1 Background, Objectives and Procedures

One of the best-identified crystallisation methods for proteins on Earth and in space is the contra-diffusion technique that has been invented and developed by the team of Professor Garcia-Ruiz of the Granada University. With this technique, the protein solutions are placed in capillaries and the crystallisation occurs thanks to a precipitating solution that progressively diffuses along the capillaries. As the capillaries are small, the monitoring requests microscopic visualisation that leads to a very limited depth of focus. The result is that the direct observation of crystals in capillaries can give rise to unfocused images. In order to overcome this classical limitation of optical microscopy, the Microgravity Research Centre of the University of Brussels developed a digital holographic microscope that allows for an in-depth reconstruction, by numerical means, of the samples being analysed. This instrument benefits from partial coherent illumination to eliminate the classical sources of noise inherent to the use of a laser source and provides an image quality that is comparable to the one obtained with the best optical microscopes. As the 3D refocusing is made by numerical means, the technique is very interesting for automated experiments in space because it eliminates the need to focus on the image of each crystal and it also compresses in a very important way the data to be stored. As the method is a true holographic one, it also records the optical phase information of the observed field. Therefore, this method gives an accurate measurement of the refractive index changes in the solution surrounding the crystals. For protein applications, this is a very important point as the refractive index changes around a crystal and is a direct measure of the depletion zone that is expected to have a deep impact on the crystallisation quality.

The major objective of the present experiment was to produce a detailed analysis and a quantitative interpretation of the relationship between the quality of the obtained crystals and the environment in which they are produced by the method of digital holography.
The experiment aimed to investigate the protein growth processes in weightless conditions using the counter diffusion technique, in order to measure:

- The parameters of the growing protein crystals,
- The composition changes (depletion zone) of liquid around the growing protein crystals.

Protein crystallisation is initiated when a protein solution is brought to supersaturation, most often by raising the precipitant (salt, polyethyleneglycol, organic solvent) concentration, so as to lower the protein solubility. In counterdiffusion experiments the precipitant and protein solutions are initially in different volumes, and diffuse toward each other through a separating gel layer. In PromISS experiments both capillary and full reactor geometries (Figure 2-16) were used. The length of the diffusion path through the separating layer determines the delay in the start of the crystallisation.

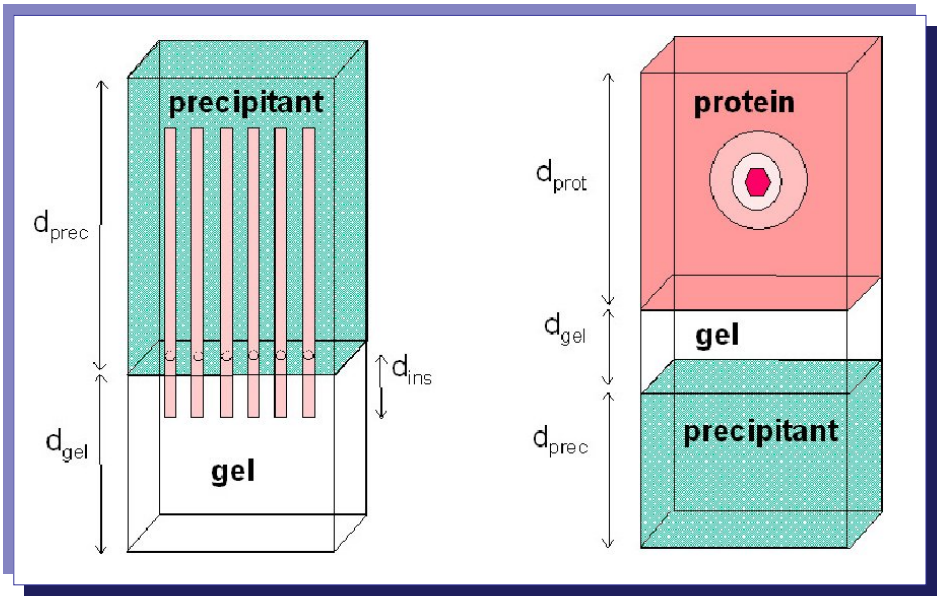

Figure 2-16: Geometry of the PromISS experiments. Internal volume of the reactors is 52 mm high, 19 mm wide, and 34 mm deep (left: capillary geometry; right: full reactor geometry)

The crystallisation experiments were observed by digital holography. This technique yields the in depth reconstruction of the field of view by numerical methods, without a mechanical focusing stage. The principle consists in the combination of the intensity and the optical phase of the object and to compute, by implementation of wave optics equations, the amplitude in planes that are parallel to the detection plane. In that way, out of focus objects can be brought into focus. Digital holography is attractive for automated experiments in space because it eliminates the need to scan the reactor, and reduces the amount of data to be stored. As the method also records the optical phase, it also gives an accurate measurement of the refractive index changes in the solution surrounding the crystals. For protein applications this is a crucial point as the refractive index is a measure of the composition of the solution.

The PromISS instrument (Figure 2-17) comprises a Mach-Zehnder interferometer where the sample is illuminated in transmission. For each image four interferograms were recorded at different phase shifts, induced by a piezo-transducer. PromISS comprised 6 experimental cells that were disposed on a rotating plate. During the experimental run, the rotating wheel sequentially placed the experimental region of interest (ROI) in the optical channel of the microscope. It was possible to define by tele-operation 6 ROI per cell. A complete rotation of the wheel was achieved in one hour. The PromISS instrument was accommodated in the Microgravity Science Glovebox (MSG) in the Destiny (US-Lab) Module of the ISS. The MSG provides power, containment, video recording, data transfer and commanding capability from ground.

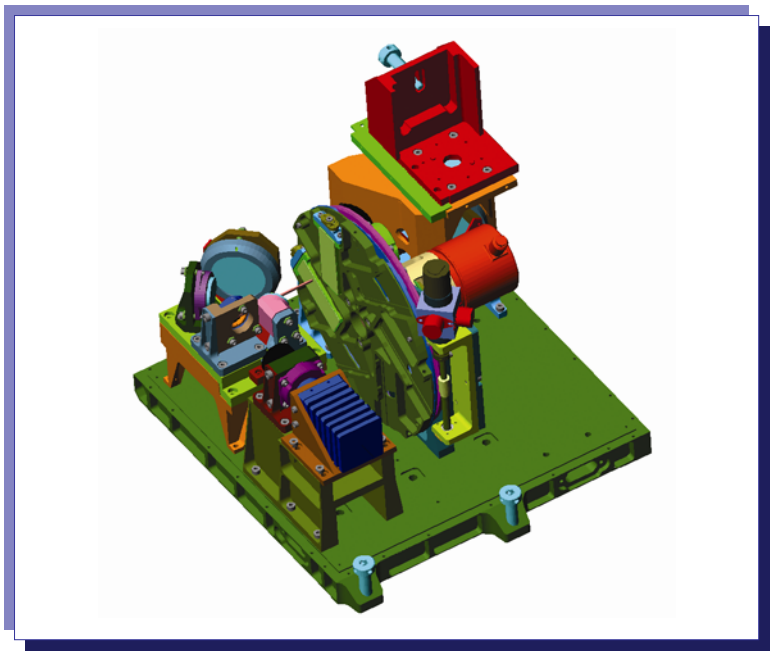

Figure 2-17: An internal view of PromISS without electronic boxes

2.3.2.1.1.2 Results

The following results are based on the first three series of PromISS experiments that have been performed: PromISS I in the context of the Belgian Soyuz mission, "Odissea" (ISS 5S mission), which took place in October-November 2002, PromISS II during the Spanish Soyuz mission, "Cervantes" (ISS 7S mission), which was executed in October 2003, and PromISS III as part of the Dutch Soyuz mission, "Delta" (ISS 8S), which took place in 2004. In each space experiment six reactors were observed in PromISS, so a total of 18 individual crystallisation experiments were performed on 6 proteins (the complex of the variable domain of a camelid heavy chain antibody with lysozyme (cablys3*lysozyme), Thermotoga marirtima triose phosphate isomerase (TIM), pike parvalbumin, hen egg white lysozyme, equine spleen ferritin, and lumazine synthase).

The data generated by the PromISS experiments consisted first in the interferometric data produced by the instrument, and second in data generated by the post-flight analysis of the quality of the crystals, and for certain experiments of the composition of the crystals and their surrounding solutions. For all three PromISS experiments

the raw interferometric data has been treated. These data consist in successive sequences of about 2.5 seconds of recording at a frame rate of 30 images/sec. In this set of 75 images, 4 images (corresponding to the four phase shift) were selected and amplitude and phase fields were computed (specific algorithms have been developed to automatically select the four images). Images have been digitalised, automatically classified, backups have been made, and amplitude and phase images (when possible) have been computed. The reconstruction in depth has been performed when necessary (crystals out of focus in the field of view). Figure 2-18 represents a numerical propagation of the optical beam on a distance of 200 µm in the direction of the optical path (an example of refocusing of an out of focus crystal is illustrated).

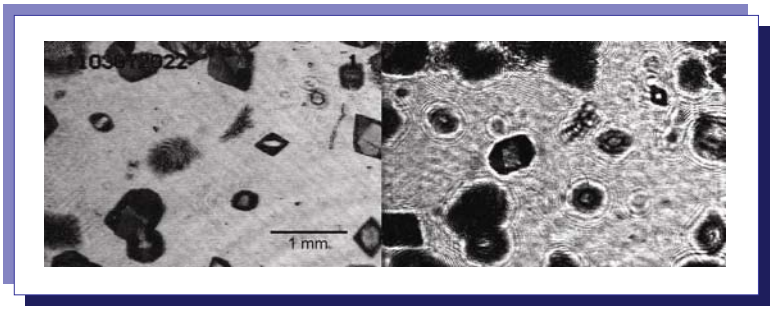

Figure 2-18: Example of amplitude computed image

Tools were developed to analyse the overall growth rate and position of the crystals. These tools are for the moment applied manually by the user. The automation of the segmentation and tracking of all the crystals contained in an image (or sequence of images) is being developed.

The first PromISS experiment was developed in less than 9 months, and performed surprisingly well in this regard, registering interferometry images every hour for the six reactors in 6 different positions. This made it possible to evaluate the moment of appearance of crystals, their growth rates, and the movement of crystals. A second instrument was built and uploaded in the context of the Spanish Soyuz Mission. This instrument has an external electronic box, and can thus be operated at the temperature of the MSG (average temperature in the PromISS II experiment 26.05 °C). The vibration sensitivity was reduced by reinforcing the holder for the beam splitter.

For 12 out of the 18 reactors that were flown for PromISS I-III, crystals were obtained in the right time frame, i.e. during observation by PromISS. In three reactors of PromISS I the high temperature caused the crystals to grow only after the end of the experiments. It was often not trivial to optimise the experiments as the timeline was very constraining. The constraint that crystals should not grow during the first five days (upload and installation of the experiments) could only be satisfied using relatively low supersaturations. On the other hand, careful analysis of the experiments showed that the use of counterdiffusion techniques reduced nucleation rates significantly for many of the proteins (TIM, parvalbumin, cablys3*lysozyme, lumazine synthase). It was thus difficult for the protein to obtain crystals before the end of the observation time. For these three proteins these problems were overcome by pre-treating protein solutions to induce intermediate phases that acted as hidden seeds. The solutions yielded crystals rapidly after supersaturation was raised, but did not produce crystals by themselves.

One of the aims of the experiments was to visualise the events occurring during a capillary counterdiffusion experiment in microgravity conditions. TIM experiments in capillary geometry were performed during PromISS I and II. Figure 2-19 shows that TIM crystals grew during the observation time and thus the crystallisation conditions and crystal growth rates could be determined.
The analysis of crystal movement showed that most TIM crystals remained stationary in the capillary during the experiment. This is important, as the aim of capillary counterdiffusion experiments is to have crystals growing in conditions that are best suited to them within a continuum of conditions created by a supersaturation wave passing through a capillary. The crystals are only weakly associated with the capillaries, as after the return of the reactors to ground the crystals could be moved out of the capillaries by induced convection. In the majority of the capillary counterdiffusion experiments few of the crystals moved.

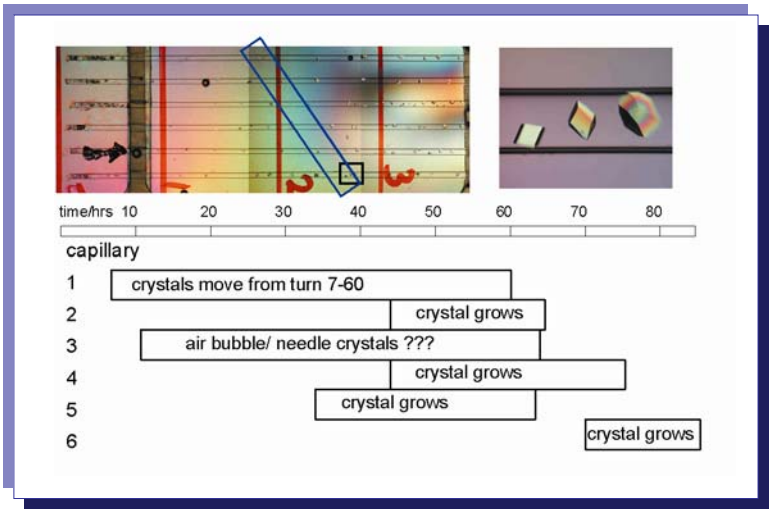

Figure 2-19: Results of an experiment with TIM in capillary geometry performed during PromISS I

Counterdiffusion crystallisation is used to effectively screen for optimal conditions of crystal growth. It requires a convection-free environment (gelled or in microgravity) that makes it possible for a precipitant wave to travel through a protein volume. It was found that for the three model proteins that were used in the PromISS experiments the use of counterdiffusion experiments resulted in crystals of different crystal forms. The difference in the outcome of the experiments can be explained on the basis of differences in the rate at which supersaturation is achieved.

2.3.2.1.1.3 Conclusions and Recommendations

The results show that counterdiffusion experiments can be useful not only for producing crystals of higher quality, but also in cases where one wants to obtain a different crystal form with improved (diffraction) properties.

The effect of diffusive conditions was very extensively investigated for the proteins TIM and cablys3*lysozyme. For TIM more than 100 datasets were collected from crystals grown either in non-convective environments (in microgravity or in gels on the ground) or by conventional techniques prone to convection. The results show that there is a clear effect of diffusion, and the crystal perfection is higher for crystals grown in a non-convective environment. Analysis of crystal growth rates and mass transport show that the depletion zone model can not explain this, as TIM crystals essentially grew in a regime controlled by surface growth rates. Diffusion is not rate-limiting and no depletion zone was formed. For other proteins like cablys3*lysozyme the influence of a diffusive environment was negligible.

Results from the PromISS experiments have shown that protein crystallisation is one of the processes found in the very complex landscape of phase behaviour of protein at high concentrations. To better understand the effect of crystal growth conditions on crystal quality requires the continued ground and microgravity research on the thermodynamics and kinetics of these processes.

2.3.2.1.1.4 Publications

1. Zegers, L. Carotenuto, C. Evrard, J.M. Garcia-Ruiz, P. De Gieter, L. Gonzales-Ramires, J.C. Legros, J. Martial, C. Minetti, F. Otalora, P. Queeckers, C. Schockaert, C. VandeWeerdt, R. Willaert, L. Wyns, C. Yourassowsky, F. Dubois, (2006), "Counterdiffusion protein crystallisation in microgravity and its observation with PromISS (Protein Microscope for the International Space Station)", *Microgravity Science and Technology, Vol. 18, Issue 3-4*, pp. 165-169

2.3.2.1.2 Study of aggregation mechanism and kinetics of ZSM-5 and Silicalite-1 nanoslabs into ZSM-5/Silicalite-1 hybrid phases under microgravity conditions (NANOSLAB)

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2.3.2.1.2.1 Background, Objectives and Procedures

Zeolites are microporous crystalline silicate materials. The applications of zeolites in catalysis, molecular separation and ion exchange processes are based on the reversible uptake of molecules and ions from the surrounding medium into the microchannels of the zeolite. The molecular mechanisms responsible for formation of zeolite materials out of monomeric or amorphous polymeric precursor materials are poorly understood. For many potential applications, optimum zeolite structures and compositions can be designed, but the knowledge is lacking on how to synthesise such tailor-made zeolites. The hypothesis of this project is that experimentation under microgravity conditions should enable considerable progress in the understanding of the formation processes of zeolite particles.

The Silicate-1 zeolite is a silicon dioxide polymorph that can be synthesised by self-organization of Silicalite-1 nanoslabs in suspension. Nanoslabs are discrete particles, measuring typically 4 x 4 x 1.3 nm, and having already the framework connectivity of the Silicalite-1 zeolite. Nanoslabs were discovered in the Team Members' laboratory, and the mechanisms and kinetics of their aggregation into Silicalite-1 crystals upon heating determined and modelled. The formation of Silicalite-1 crystals occurs via a sequence of aggregation steps of specific nano-species. Convection upon heating was observed to have a marked influence on the kinetics. Stirring of the nanoslab suspension resulted in a significant retardation of the aggregation process. Thus it was expected that microgravity would have a strong impact on the kinetics of zeolite formation.

The influence of microgravity conditions on the kinetic parameters has been studied in detail by sounding rocket experiments before missions aboard the ISS. The sounding rocket experiments were based on the post flight analysis of a series of samples of nanoslab suspensions heated for different time-spans before quenching. This way the complete aggregation sequence from nanoslabs to crystalline material could be studied. To ascertain the observed effects due to microgravity, on ground reference experiments under identical conditions using the same hardware were performed. A strong retardation of the aggregation rate of the nanoscopic entities was observed under microgravity as compared to gravity. This unexpected result led to the discovery of a liquid-crystal-like phase of the nanoslabs, which seems to be essential to enable aggregation.

The objectives of the NANOSLAB experiment were threefold:

1. Confirmation of the microgravity effect previously observed during the sounding rocket missions: During the sounding rocket missions a retardation of aggregation as a function of anisotropy of the particles and absence of gravity was observed. To confirm the accuracy of these findings additional microgravity experiments were called for.
2. Study of the temperature dependency of the microgravity effect: Due to the short duration of sounding rocket experiments (minutes) the experiment temperatures needed to be very high (T>150°C) to assure completion of the crystallization. A microgravity experiment at lower temperature with longer duration was necessary to find out if the microgravity effect is temperature dependent and, if so, in what manner.

3. Study of the effect of charge on the ordering of nanoslabs under microgravity conditions: By variation of the nanoslabs' composition, extra charge can be incorporated into the particles. It was expected that this extra charge not only has an impact on the ordering kinetics in general but also affects the microgravity effect. Comparison of the aggregation kinetics of the modified nanoslabs with the set of kinetic data of the native nanoslab aggregation was considered to be a suitable strategy to study the effect of extra charge on nano-particle ordering.

The NANOSLAB hardware consisted of a sample unit and an electronic box. The sample unit served to accommodate 30 cells, assembled in 10 cartridges of 3 cells with 1.5cc volume contained by 3 levels of containment each. The unit also provided a frame wherein the 10 cartridges were mounted, together with a top cover and bottom cover plate. The electronic box contained 3 Printed Circuit Boards (PCB). This hardware was designed to be installed and operated within the Microgravity Science Glovebox (MSG) onboard the International Space Station (ISS).

After filling the cartridges, launch and transfer to the ISS, Nanoslab was planned to be installed inside the MSG, running autonomously once switched on. The samples were to be grouped in ten cartridges of three cells each. Cartridges were to be heated actively and sequentially to the process temperature (95°C) at intervals of 4.8 hrs. Quenching was foreseen to occur for all 10 cartridges at the same time by pressing them down onto the cold-plate available in the MSG. The samples then had to be returned to ground for analysis. No data-downlink was necessary, readouts of the temperature sensors of the samples' environments was saved and stored. An independent temperature logger in the experiment was necessary to assure samples always were within acceptable temperature ranges during transport and stowage (below 28°C).

2.3.2.1.2.2 Results

In the first activation during the Belgian Soyuz mission, "Odissea" in October-November 2002 the LED "ON" on the electronic box did not operate. The foreseen contingency measure of re-starting took no effect. Checking if the heating sequence had started (possible LED failure only) resulted in the observation that the experiment had failed.
This caused the decision to return the electronic box instead of the sample unit to pinpoint the reason for the malfunction. It turned out that an electronic component was damaged during launch/transport preventing the electronic box from functioning.
A successful re-flight of the experiment took place in October 2003 during the Spanish Soyuz mission "Cervantes".

The samples obtained were of excellent quality and the soundness of the experiment strategy and the hardware has been fully proven. Also it could be verified that the quenching of the aggregation process was successful and that the obtained samples were stable during analysis.

In all microgravity experiments it was observed that microgravity significantly retarded each individual aggregation step. The influence of microgravity is not constant over the whole aggregation process. The less anisotropic the particles, the less their aggregation rates are affected by reduced convection.

1. Confirmation of the microgravity effect previously observed during the Maxus missions
   A strong microgravity effect was observed confirming the sensitivity of the studied systems to convection and shear forces.
2. Study of the temperature dependency of the microgravity effect
   Comparison of the aggregation kinetics at 95°C and 155°C resulted in the observation that reduced temperature enhance the microgravity effect considerably. Also it was found the most retarded step at 95°C is linked to the presence of plate-like particles with the most pronounced aspect ratio.
3. Study of the effect of charge on the ordering of nanoslabs under microgravity conditions
   Charge has a pronounced effect on the aggregation of nano-species in the studied system. Under microgravity conditions this effect is even further enhanced.

These findings are spectacular as they are inexplicable in the view of isolated particles approaching and aggregating. Only the formation and presence of a liquid-crystal like ordered phase before aggregation can

account for the observations. An extensive on-ground study by now has confirmed the occurrence of those Ordered Liquid Phases (OLPs). The microgravity experiments revealed the strong impact of convection and shear forces on the kinetics which indicates the important role the OLPs play during the studied zeolite formation. The discovery of the OLPs not only is useful for optimization of zeolite synthesis but can also be used to design hierarchical, functional materials as has already been shown.

2.3.2.1.2.3 Conclusions and Recommendations

The ex-situ studies of the crystallization kinetics of the Silicalite-1 type zeolite have revealed a previously unnoticed aspect of the formation of zeolites with structuring agents. The strategy to prepare samples under microgravity conditions and study them at leisure has fully met its expectations and should be considered as a worthwhile option whenever systems are susceptible to quenching. The biggest advantage of this strategy is the possibility to prepare a large number of samples increasing the reliability of the observables. However, the discovery of the OLPs now makes microgravity in-situ observation and study necessary as there is up to now no way to conserve the status quo of the liquid-crystal-like assemblies in the system at given times. For this microgravity project the combination of ex-situ screening with the detailed in-situ analysis of selected systems seems to be the most promising approach.

2.3.2.1.2.4 Publications

Not applicable.

3 ACRONYMS

AGEING	Effects of the gravity altered environment on Drosophilia motility, behaviour & ageing
BAND	Banding
BDC	Baseline data collection
BPV	Blood pressure variability
BRS	Baroflex sensitivity
CARDIOCOG	Cardiovascular adaptation to weightlessness
CHROMOSOME	Chromosomal aberrations in blood lymphocytes of astronauts
CNS	Central nervous system
CSA	Canadian Space Agency
DNA	Deoxyribonucleic acid
E	Epinephrine
ECG	Electrocardiogram
EEA	Erasmus Experiment Archive
EEG	Electroencephalogram
ERP	Event related potential
ESA	European Space Agency
ESF	European Science Foundation
EVA	Extra Vehicular Activity
EVP	Event related potential
FISH	Fluorescence in-situ hybridisation
FO	Flight operations; Functional objective
HDBR	Head down bed rest
HDT	Head down tilt
HRV	Heart rate variability
IFOC	ISS Flight Order Contract
ISS	International Space Station
IU	International units
JAXA	Japan Aerospace Exploration Agency
LED	Light-emitting diode
MASER	MAterial Science Experiment Rocket
MESSAGE	Microbial experiment on Space Station about gene expression
MSG	Microgravity Science Glovebox
M-VEP	Motion-onset related visual evoked potential
NASA	National Aeronautics and Space Administration
NE	Norepinephrine
NeuroCOG	Directed attention brain potentials in virtual 3-D space in weightlessness
OI	Orthostatic intolerance
OLP	Ordered liquid phases
PCB	Printed circuit board
PromISS	Counterdiffusion protein crystallisation in microgravity and its observation with the Protein Microscope for the ISS
ROI	Region of interest
Roscosmos	Russian Space Agency
SE	Standard error
SYMPATHO	Sympathoadrenal activity in humans during spaceflight
TEXUS	Technologische EXperimente Unter Schwerelosigkeit
TIM	Thermotoga marirtima triose phosphate isomerase
US	United States
VEP	Visual evoked potential
ZARM	Zentrum für Angewandte Raumfahrt Microgravitation