Thursday, June 12

Session JP4
Room 4
2:30 - 5:30 p.m.

Biological Bases of Space Radiation Risk

Hematopoiesis Dynamics in Irradiated Mammals: Mathematical Modelling

O. A. Smirnova

Research Center of Spacecraft Radiation Safety
Shchukinskaya st., 40, Moscow 123182, RUSSIA

Mathematical models were developed which describe the dynamics of the bone-marrow hematopoiesis in mammals exposed to acute and chronic radiation. The blood-forming system is represented here as a combination of major hematopoietic lines: thrombocytopoiesis, lymphopoiesis, erythropoiesis, and granulocytopoiesis. The models are based on theories and facts well known in radiobiology and hematology. The models consist of the systems of nonlinear differential equations. Concentrations of cells of the above-indicated hematopoietic lines serve as variables and the dose of acute and the dose rate of chronic irradiation are variable parameters in them.

Analysis has shown that the models can quantitatively describe the effects of acute and chronic exposures on the hematopoietic system of mammals (mice and rats). The models simulate the dynamics of damage and recovery of pools of mature blood cells and their precursors in the bone marrow after acute irradiation, describe depletion of individual hematopoietic lines during chronic exposure at high dose rates, and reproduce the ability of the hematopoietic system to adapt itself to protracted irradiation at low and moderate dose rates. The models also describe the experimentally observed paradoxical effects of low level exposures. The most important of them are the stimulation of adaptive processes in the lymphopoiesis and granulocytopoiesis at low dose rates of chronic irradiation and the decreasing of radiosensitivity of thrombocytopoiesis and granulocytopoiesis systems in the result of prolonged exposure at low doze rates. The models can be useful for prediction of hematopoiesis system response on irradiation during long-time space mission.

The work was funded in part by DNA/AFRRI (USA).

ESTIMATING HEALTH RISKS IN SPACE FROM GALACTIC COSMIC RAYS

Francis A. Cucinotta[1], J.W. Wilson[1], J.F. Dicello[2], J. R. Williams[2], and Mack Mabry[2]
[1]NASA, Langley Research Center, Hampton VA 23681-0001
[2]The Johns Hopkins Oncology Center, 600 Wolfe St., Baltimore MD 21287-5001

Long term space travelers to the moon or Mars will experience protracted exposures to galactic cosmic rays (GCR). The GCR consist of high energy hydrogen, helium, and heavy ions for which there are few epidemiological data on expected carcinogenic risk. Improvement in the understanding of the risk from the GCR will require better understanding of radiation physics, radiation shielding effectiveness, and basic studies in cancer biology. Radiation physics and shielding studies are currently hampered by the lack of fundamental understanding of the risks of cancer attributable to ions. These data are necessary to design cost effective shielding for space travelers. We present an overview of computational models that describe the passage of GCR through spacecraft shielding and tissue and highlight the importance of track structure effects. We discuss efforts to model molecular interactions of proto-oncogenes and tumor suppressor genes known to be important in human cancers and discuss critical experiments.

The HZETRN (high charge and energy ion transport) model describes the passage of the GCR through shielding and tissue. Methods to validate HZETRN include ground-based experiments of nuclear interaction and transport properties of neutrons, and light and heavy ions. Comparison of the HZETRN code to measurements on the Shuttle and the MIR space station provide further means for validation. However, the understanding of optimal shielding materials for lunar/Mars spacecraft relies on biological response models. We discuss shield evaluation using conventional risk assessment and track structure models that describe radiobiology experiments with ion beams for DNA strand breaks, HPRT mutations, and tumor prevalence in mice. The understanding of track structure effects is expected to be a determining factor in shielding selection, however existing studies are limited by their relevancy for estimating cancer risk in humans.

We are developing computational models in parallel with primary human cell lines that quantitatively describe relevant molecular pathways that are genetically or epigenetically altered in the progression towards human carcinogenesis. We have developed a non-linear kinetics description of cell cycle progression and inhibition by cyclins, cyclin dependent kinases (cdk), pRb, p107, E2Fs, and cdk inhibitors (cdki). The model is extended to consider ras and raf signal transduction and downstream effects on cdks and cdkis. Radiation exposure alters cellular functions through gene mutation and activation of signal transduction pathways. We discuss calculations of p53 signal transduction following DNA damage as a function of radiation type to study alterations in cellular proliferation. Calculations for photons and heavy ions are compared to limited existing measurements. Computer simulations of modifications of cellular proliferation by gene mutations induced by photons or heavy ions in relevant molecular pathways are discussed. These preliminary studies are being used to suggest critical experiments to evaluate the effectiveness of heavy ions and to establish the role of counter-measures such as radiation shielding in reducing cancer risks.

FAILURE OF HEAVY IONS TO AFFECT PHYSIOLOGICAL INTEGRITY OF THE CORNEAL ENDOTHELIAL MONOLAYER.

Jan P. Koniarek and Basil V. Worgul
Department of Ophthalmology, Eye Research Division, Columbia University, New York, NY 10032

INTRODUCTION

As planning for the space station and voyages to the planets is coming to an advanced stage, there is an increased interest in effects of heavy ion radiation on biological systems, especially on human health. Prolonged habitation in space will expose astronauts to this type of radiation from cosmic rays. Since it will not be possible to shield them from such highly energetic radiation, predicting their effects and designing appropriate therapeutic countermeasures is of great importance to mission planners.

It has long been recognized that heavy ions cause damage to living organisms at the molecular, cellular, and tissue levels. Furthermore, it has also been proposed, based on morphological observations, that they generate "microlesions" - discrete regions of focal cellular destruction (e.g., P. Todd. *Adv, Space Res.* 3:187-194, 1983). In membranes these microlesions would show up as holes. Several morphological studies conducted to test this hypothesis seem to suggest such an effect, but other studies cast doubt on this theory (Worgul et al., *Adv. Space Res.* 10: 315-323, 1989). A study investigating the effect of heavy ions on physiological parameters of tissues *in vitro* failed to show any effects (Koniarek and Worgul *Adv. Space Res.* 12: 417-420, 1992).

To determine if microlesions can form, and if they produce a prolonged physiological effect on cells, we investigated the effects of heavy ions on the electrical potential difference that is generated across the monolayer of cells of the corneal endothelium. The transendothelial electrical potential difference (TEPD) is generated by the endothelial fluid pump as it maintains the cornea at the level of hydration required for transparency. This fluid pump is driven by the transport of electrolytes across the endothelium. A perfectly intact endothelial cell layer is needed to maintain the TEPD at its normal level. Should holes in cell membranes develop, the ionic composition inside and outside would be rapidly altered and the TEPD would be abolished; hence this parameter is an excellent indicator of tissue integrity.

We also developed mathematical models related to formation and closure of holes in corneal endothelial cell membranes, taking into account parameters such as diffusion of phospholipids and attenuation of this diffusion by intercellular components and the extracellular matrix of these cells.

METHODS

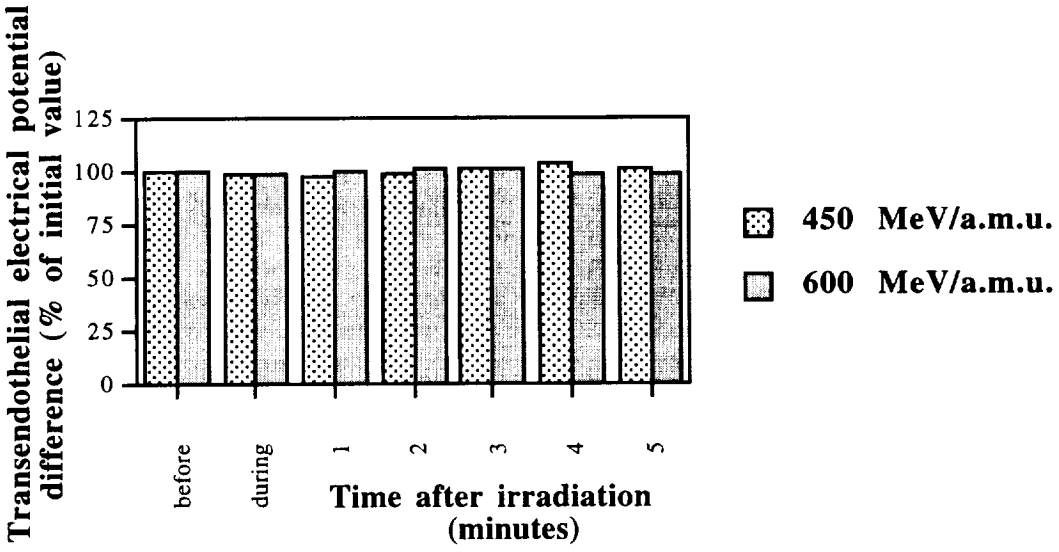
Corneas were dissected from RCS rats. Their epithelial layer was scrapped off (since this layer itself is also a source of a potential difference), and the stroma with its intact endothelial monolayer was mounted in an experimental chamber filled with a solution of basic salts and glucose, and which was temperature controlled at 37^0C. The TEPD of about 600-800 μV was monitored with a pair of electrodes, one on each side of the preparation (Koniarek et al., *Invest. Ophthalmol. Vis. Sci.* 29: 657-660,1988).

While the TEPD was monitored, the corneas were irradiated perpendicular to the beam path by one Gy doses of ^{56}Fe ions of 450 and 600 MeV/a.m.u. (LETs = 195 and 173 keV/μm respectively). This ion is among the most prevalent in the zoo of particles in galactic cosmic rays, and it has been suggested that these particles could produce microlesions. The ions were delivered for 0.5 to several minutes at the rate of 10^3 particles/mm^2/sec.; thus a typical endothelial cell (25 μm in diameter) was traversed by about 16-18 heavy ions per second. The experiments were conducted by the BEVALAC compound accelerator in Berkeley, California, before its decommissioning.

RESULTS

Before the irradiation the TEPD was monitored for about 30 minutes to ensure that it was steady. During irradiation the TEPD did not change significantly from pre-irradiation values, except for a transitory artifact resulting from the interaction of the ion beam and the recording electrodes, which ceased as soon as the beam was turned off. After irradiation the TEPD was monitored for 5 minutes (and in some cases longer, up to 15

minutes), and its values were not statistically significantly different from their pre-irradiation levels. The graph below summarizes our results.



CONCLUSION

Our results obtained with 450 and 600 MeV/a.m.u. ^{56}Fe ions failed to show any effects of this radiation on the TEPD. This observation suggests that no physiologically significant microlesions in form of overt, stable holes were produced in the cell membrane. It is also possible that membrane holes were in fact produced during irradiation, but that they were extremely transitory, and that they were quickly eliminated by the reforming cell membrane; our theoretical models indicate that such rapid repair is possible within a fraction of a second. In either case the several ion hits per second per cell are not enough to cause any long-lasting physiological effects on cell integrity and function of the corneal endothelium.

APPLICATION OF AN UNBIASED TWO-GEL cDNA LIBRARY SCREENING METHOD TO EXPRESSION MONITORING OF GENES IN IRRADIATED VERSUS CONTROL CELLS

E.K. Balcer-Kubiczek[1,4,5], S.J. Meltzer[2,3,4,5], L.-H. Han[1], X.-F. Zhang[1], M.-S. Zhong[1], G.H. Harrison[1,5], and J.M. Abraham[2,4,5]

[1]Department of Radiation Oncology (Radiation Research Division), [2]Department of Medicine (Gastroenterology Division), School of Medicine, [3]Veterans Affairs Hospital, [4]Molecular and Cell Biology Program, Graduate School, and [5]Program in Oncology, Marlene and Stewart Greenebaum Cancer Center, University of Maryland, Baltimore, MD 21201

INTRODUCTION

Rapid alterations in gene expression can occur after cellular exposure to injurious agents, including various types of ionizing radiation, ultraviolet light, 12-*O*-tetradecanoyl phorbol-13-acetate (TPA), heat shock, hydrogen peroxide and hypoxia. More than 50 genes responding to two or more of these agents have been described. Of these, fewer than 10 genes (for example, *gadd45*, *WAF-1/Cip1*, *mdm2*) respond more to DNA-damaging agents such as ionizing radiation than to non-damaging agents such as TPA. The discovery of radiation-responsive genes provides a starting point for elucidating the molecular processes associated with radiation pathology. A detailed understanding of the pleiotropic effects of ionizing radiation will require implementation of sophisticated methods for gene expression analysis and gene discovery.

In several studies, subtractive hybridization and differential display of messenger RNA (mRNA) have been employed to identify new genes activated by ionizing radiation. One of us (JMA) recently developed an improved nonbiased polymerase chain reaction (PCR)-based method for the identification of new genes or complementary DNA (cDNA) sequences by *random* differential library screening. In this paper, we describe a modification of this method, termed *the unbiased two-gel cDNA library screening method*, and its application to comparing transcriptional profiles in control *versus* irradiated cells, resulting in several important results.

METHODS

Our procedure involves PCR amplification of insert cDNAs pooled in a 12 x 16 matrix, using primers complementary to vector sequences flanking the cloning site followed by electrophoresis on exact duplicate agarose gels. The associated duplicate Southern blots were probed with cDNA synthesized from mRNAs isolated from control *versus* irradiated cells, and the autoradiographs compared for differential expression of the amplified cDNA sequences. Lanes that displayed differential expression patterns under given experimental conditions were further characterized using a second round of Southern blot analysis, followed by sequencing. Differential expression was confirmed by Northern blot analysis.

Two cDNA libraries constructed in the Lamba-ZAP-II vector, one from HL60 cells and another from MCF7 WT cells were used to prepare exact duplicate cDNA arrays for screening. Bacterial transformants were obtained by infecting *E.coli* strain XL1-Blue MRF'. Bacteriophage colonies were picked at random and propagated in 200-μl aliquots, and inserts (0.3-2.0 kb) were amplified by PCR using appropriate primers. Investigated conditions, doses, radiation type and (post-irradiation time-points), for the initial identification of ionizing radiation-regulated genes were: 20 Gy X rays (3 h), 1.2 Gy fission neutrons (3 h or 1 week); 2 Gy 1 GeV/n Fe-ions (1 week; study currently in-progress). Our initial studies involving X-irradiation and short post-irradiation time of 3 h were performed with pre-leukemic HL60 cells; these cells are null for *p53*. All the other work was done using epithelial MCF7 WT cells; these cells are normal for *p53*. To explore a signalling pathway distinct from the radiation response, conventional Northern analysis was used to examine selected gene transcription after TPA and forskolin treatments. Chemical-treated or control cells were harvested, lysed, and used as a source of mRNA. Northern hybridization was done using the radioactively labeled cDNA corresponding to a the gene of interest.

RESULTS

The aim of this study is to identify genes involved in radiation response at the cellular level, including neoplastic transformation. By screening approximately 3000 cDNA clones, we isolated to date (January 1997)

13 independent clones differentially expressed in HL60 and MCF7 WT cells.

HL60 cells have been characterized as radiation-sensitive (D_0 < 1 Gy). Exposed to 20 Gy of X rays, they were viable for up to 12 h. Of 5 candidate radiation-responsive genes, the expression of one gene, *Csa-19*, was characterized in detail. The abundance of *Csa-19* mRNA deceased dramatically in HL60 cells 3 h after X-irradiation. The same transcription pattern was observed in MCF7 WT cells, and after exposure of HL60 or MCF7 WT cells to 1.2 Gy of fission neutron-irradiation. Our result that *Csa-19* is similarly repressed in *p53* normal and abnormal cells provides a new example of an immediate-early gene that is transcriptionally independent of *p53*, in contrast to other previously-discovered radiation-responsive genes (such as for example, *gadd45*, *WAF-1/Cip1*, *mdm2*).

Two of three known genes isolated in fission neutron-irradiated MCF7 WT cells, *L-23*, *κ-casein* and *TI-227* 1 week post-irradiation have been reported by other groups in association with tumorigenicity and metastatic potential. Accordingly, the L-23 gene is implicated in *genomic imprinting* process and, thus, could be involved in multigene human diseases such as breast cancer. The postulated cellular function of *TI-227* is to regulate the expression of various genes as a transcription factor in the complex process of metastasis. All identified genes were nuclear, except oxidase II (mitochondrial). For remaining genes, no match has been found in the public data bases. Attempts to characterize both novel and known genes discovered in this laboratory is currently underway.

CONCLUSION

The present method offers several advantages over other approaches to expression analysis. The parallel format of the assay provides a *simultaneous differential expression* output for approximately 200 genes. This contrasts with sequencing-based methods, which require serial data collection for expression analysis. The availability of commercial cell-specific libraries provides a rich and readily accessible resource of human cDNA clones for this assay. Once the arrays have been constructed, they can be re-used for gene expression monitoring in different radiation conditions. As demonstrated, other practical advantages of this method are the ability to confirm differential expression by Northern blotting, the capability to detect low-copy-number transcripts, and the immediate availability of full-length cDNA clones for further analysis and sequencing. The identification of several known and possibly novel ionizing radiation-regulated genes in human pre-leukemic HL60 cells and in breast carcinoma MCF7 WT cells demonstrate sensitivity of an unbiased two-gel cDNA library screening method. Our current method provides a rapid and efficient means for large-scale discovery of human genes regulated by ionizing radiation.

Supported in part by the National Aeronautics and Space Administration (NASA) grant NAGW-4392.

DETECTION OF RADIATION-INDUCED DNA STRAND BREAKS IN MAMMALIAN CELLS BY ENZYMATIC POST-LABELING

Shunji Nagaoka[1], Yoshitaka Taniguchi[2], Sumiyo Endo[2], Takuo Onizuka[2], Masahiko Hirano[2], Kazunobu Fujitaka[3], and Takeo Ohnishi[4]

1) National Space Development Agency of Japan, Tsukuba, Japan, 2) Toray Res. Center, Inc., Kamakura, Japan, 3) National Institute of Radiation Sciences, Chiba, Japan, 4) Nara Medical Univ., Kashihara, Japan

INTRODUCTION

An aim of this study is to develop a *in-situ* analytical method of DNA damage of human and animal cells caused by cosmic rays in space. It may be important to analyze DNA damages in cellular level to evaluate biological effects of cosmic radiation. In this study, we developed novel systems to detect DNA-strand breaks in mammalian cells by enzymatic post-labeling. In this system, we apply catalytic reactions of enzymes known as E.coli DNA polymerase I and terminal nucleotidyl transferase (TdT)) which recognize 3' termini of DNA and introduce nucleotides to the sites.

METHOD

SV40-transformed human fibloblast cell line, WI38VA13, its non-transformed counterpart, WI38, and human glioblastoma cell line, T98G were used. Irradiated cells were chemically fixed, and then subjected to the post-labeling procedure. The reaction mixture contained enzyme (DNA polymerase I or TdT) and substrate (nucleotide). Tritiated nucleotide ([^{3}H] -dATP) was used to detect DNA strand breaks by autoradiography or using a liquid-scintillation counter. In the autoradiography, grains induced by [^{3}H] -dATP molecules in individual cell could be detected visually under a light microscopy. Grain numbers per nucleus were counted subtracting the background grains with using non irradiated cells. In the liquid-scintillation counting method, the total [^{3}H] -dATP uptake was measured under homogenate condition.

X-ray irradiation was carried out on ice using a MBR-1520 (Hitachi Medico) instrument operating at 150 kV and 20 mA. Carbon ion irradiation (290 MeV/n, LET around 100 KeV/μm) was carried out using Heavy Ion Medical Accelerator (HIMAC) at National Institute for Radiological Sciences at Chiba.

RESULTS

To optimize a detection sensitivity, conditions for chemical fixation and post-labeling were examined. Fixation with 1% formaldehyde followed by 70% ethanol did not cause non-specific DNA strand breaks. Cells were, however, mostly shrunk and changed morphologically. This changes reduced efficiency to detect grains in autoradiography. On the other hand, non-specific DNA strand breaks were caused with fixing by methanol. It may be due to endogenous endonuclease or radicals, but no cell morphology change was observed. Grains found in the nuclei were more clearly detected than fixed with formaldehyde.

In the post-labeling reaction, a combination of enzyme and substrate concentrations was found to be critical to attain sufficient sensitivity. We confirmed that [^{3}H]-dATP uptake was increased depending on both enzyme and substrate concentrations and also their reaction time.

In the preliminary experiments with X-ray irradiation, the autoradiographic method developed with methanol as a fixative can detect DNA strand breaks of cells exposed 40 Gy and 100 Gy by DNA polymerase I and TdT, respectively.

We also applied the system with DNA polymerase I to cells irradiated by carbon ions. Cells exposed to 5 Gy showed significant increase in the grains than non-irradiated one. The grain number significantly increased in the dose dependent manner up to 40 Gy. Interestingly, the grain number per cells irradiated with 80 Gy was less than that irradiated with 40 Gy. Since DNA polymerase I can only recognize 3' termini in the presence of single-strand DNA templates, the result obtained at the high dose of heavy ions exposure suggested double-strand breaks or deletions of DNA rather than single-strand breaks.

EVALUATION OF BLEOMYCIN-INDUCED CHROMOSOME ABERRATIONS UNDER MICROGRAVITY CONDITIONS IN HUMAN LYMPHOCYTES, USING "FISH" TECHNIQUES

[1]Mosesso P., [2]Schuber M., [2]Seibt D., Schatz A., [1]Fosci A., [1]Fonti E. and [1]Palitti F.
[1]*University of Tuscia, Dept. of Agrobiology and Agrochemistry, 01100 Viterbo, Italy.*
[2]*DLR Institut of Aerospace Medicine, MUSC, 51140 Köln, Germany.*

ABSTRACT
One way to improve the estimation of radiation risks to human beings in future space mission and for establishing radiation standards for man in space is, in addition to physical dosimetry, to obtain quantitative information regarding the effectiveness of space radiations to induce chromosomal damage under microgravity conditions, which appear to influence in terms of potentiation the genetic effect.
Mosesso et al., 1996 reported that treatments with the radiomimetic agent bleomycin performed under simulated microgravity conditions in human lymphocytes using the cuvette clinostat as a tool to simulate weightless conditions induced significant increases of aberrant cells bearing dicentric chromosomes, as detected by conventional cytogenetic analysis, compared to the parallel treatments performed on the "ground".

In the present study we aim to extend the results obtained in the above mentioned investigation evaluating the induction of chromosomal damage with fluorescent *in situ* hybridisation (FISH) and chromosome-specific composite DNA probes (chromosome painting) to detect stable aberrations such as reciprocal translocations which can persist during several cell divisions and complex aberrations.
This approach will permit us to enhance the power of cytogenetic analysis and correlate phenomena of mis-repair of DNA with the eventual presence of complex aberrations which cannot be detected with conventional methods.

REFERENCES
Mosesso P., Schuber M., Seibt D., Fiore M., Fonti E., Gigliozzi S. and Palitti F.(1996) Effect of simulated microgravity conditions on radiation-induced chromosome aberrations and DNA-repair in normal human lymphocytes Proceedings of sixth European Symposium (European Space Agency ESA SP -390).

ACKNOWLEDGEMENTS
This investigation is sponsored by Italian Space Agency (ASI), Contract No. RS113.

TECHNICAL DESCRIPTION OF THE SPACE EXPOSURE BIOLOGY ASSEMBLY SEBA ON ISS

P. Hofmann[1], P. Rank[1], J.U. Schott[2], H. König[3]
[1]Kayser-Threde GmbH, Wolfratshauser Str. 48, D-81379 München; [2]DLR, Linder Höhe, D-51147 Köln;
[3]ESA/ESTEC, Keplerlaan 1, NL-2201 AZ Noordwijk

ABSTRACT

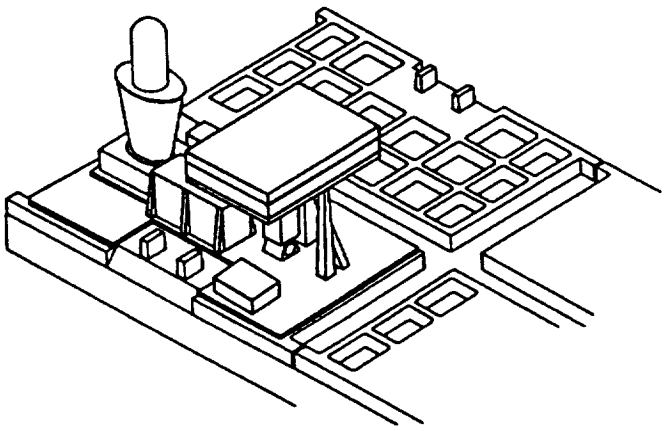
The "Space Exposure Biology Assembly" (SEBA) is a new multi-user facility for future space experiments in the fields of exobiology and radiation research. SEBA is scheduled for flight on the EXPRESS Pallet, an external platform of the International Space Station, in the year 2001.

The SEBA facility is dedicated to experiments in areas such as photobiology, photoprocessing and dose depth distribution measurements of space radiation. Most experiments require extended duration of space environment. The facility design of SEBA has been developed from concepts of already existing and successfully flown exposure facilities for microbiological research in space. New concepts for dosimetric measurements shall be realized.

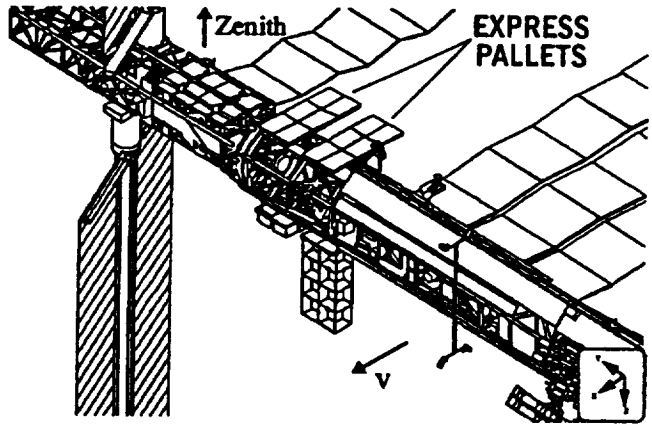
SEBA MECHANICAL CONFIGURATION ON EXPRESS PALLET

The configuration of SEBA on the EXPRESS Pallet is shown below. The SEBA facility is planned to be composed of two independent experiment units (EXPOSE used for photobiology, photoprocessing and Matroshka, used for dose depth distribution measurements of space radiation), a pointing device used in conjunction with EXPOSE, a central Control and Power Distribution Unit, a mechanical support structure as interface to the EXPRESS Pallet, an interconnecting harness, and thermal hardware. The remaining free areas on the carrier platform will be reserved for self-standing add-on experiments. The envelope of SEBA is defined by the footprint of a single EXPRESS Pallet Adapter (ca. 1.20 x 1.05 m²); the height will maximally be ca. 1.25 m.

The actual SEBA configuration as a result of the ongoing phase B study shall be presented in some detail, and its scientific capabilities shall be highlighted.



Preliminary configuration of SEBA



Section of the truss of the International Space Station with attached EXPRESS Pallets

Cytogenetic Research in Biological Dosimetry

V.Shevchenko[1], G.Snigiryova[2], V.Petrov[3], B.Fedorenko[3], S.Druzhinin[3]

[1] Institute of General Genetics, Russian Academy of Sciences

[2] Moscow Research Institute of Diagnostic and Surgery, Ministry of Health and Medical Industry of the Russian Federation

[3] State Scientific Center of RF - Institute for Biomedical Problems, Moscow

The possibility of using cytogenetic methods for biological dosimetry is explored on the basis of the materials from examinations of people affected as a result of the Chernobyl accident, nuclear explosions at the Semipalatinsk testing site, radioactive contamination of the Techa river (Chelyabinsk region) and the accident at the Three Miles Island nuclear plant (Pennsylvania, USA).

The level of lymphocytes with unstable chromosomal aberrations has been found to reduce in time. However, despite a relatively long time from the moment of irradiation (years, decades) in all examined groups of people this index significantly exceeds the control level.

The most promising for the purposes of biological dosimetry is the estimation of the frequency of stable chromosome aberrations (symmetric translocations) by the FISH method.

The preliminary data obtained from the cytogenetic examination of 23 astronauts were presented in this work too. Cytogenetic examinations included the analysis of unstable chromosome aberrations before and after a space flight. The investigations in the pre-flight period have been carried out with 14 of the astronauts. In this case the obtained results were assumed as the spontaneous level. The frequency and types chromosome aberrations were studied in 11 astronauts after the first space flight and in 11 astronauts - after repeated flights.

It is ascertained that within the pre-flight period the level of chromosome aberrations little differs from the assumed background values. The frequency of chromosome aberrations and aberrant cells raises after the flights. In particular, the frequency of dicentrics and centric rings was more than 6 times as high as the background level after the first space flights, and more than 15 times - after repeated flights.

The problems concerning the application of cytogenetic methods to estimate absorbed doses.