Radiobiological Studies
at the Joint Institute for Nuclear Research

Pavel Bláha
pavel.blahax@gmail.com
Laboratory of Radiation Biology

- **1959.** First radiobiological experiments at JINR – comparative evaluation of effects of protons and gamma on laboratory animals
- **1964.** Stationary laboratory opened at the Laboratory of Nuclear Problems (LNP)
- **1978.** Establishment of the Biological Research Sector at the LNP – main aim: differences in the biological effectiveness of ionizing radiation with different physical characteristics
- **1988.** Establishment of the LNP’s Biophysics Department
- **1995.** Establishment of JINR’s Department of Radiation and Radiobiological Research
- **2005.** Establishment of JINR’s Laboratory of Radiation Biology
Irradiation possibilities

- Gamma sources
  - Rokus-M ($^{60}$Co)

- Protons

- Heavy charged particles
Phasotron (Synchrocyclotron) - LNP

Protons with energies up to 660 MeV – around the peak of the GCR protons
U-400M - LNR

Heavy ions

$^7\text{Li}^2+ \rightarrow ^{86}\text{Kr}^9+$

Energy around 50 MeV/nucleon
The LRB special stationary setup “Genome” at the U-400M

Fast automatic irradiation of thin biological samples (or small volumes of suspension) with high LET heavy ions in a wide range of absorbed doses
Synchrophasotron - LHEP

- Synchrophasotron, an accelerator built in Dubna in 1957, has become the biggest and the most powerful for his time. Its magnet weighs 36000 tons and is registered in the Guinness Book of Records as the heaviest in the world.

1957 - 2003
Acceleration of protons (up to 10 GeV/nucleon) and heavy ions
Nuclotron - LHEP

Heavy ions up to $^{197}\text{Au}^{79+}$
Energy: 0.6 – 4.5 GeV/amu
Future: Booster + NICA

Superconducting accelerator complex NICA
(Nuclotron based Ion Collider fAility)

Fixed target experiments area (b.205)
Extracted beams from Nuclotron

KRION-6T and HILac (3.5 MeV/u)

SPP and LU-20 (5 MeV/u)

Nuclotron 0.6-4.5 GeV/u

Cryogenics

NICA Collider (1-4.5 GeV/u, C~500 m)

2nd IP

HV e-cooler

Booster (3-660 MeV/u) inside Synchrophasotron yoke

Multi-Purpose Detector (MPD)
GCR and JINR’s accelerators energy spectra

Nuclotron range
U-400M range
Booster range
Why radiation? - Effects of ionizing radiation

- Ionizing radiation is an **extraordinarily** efficient agent for causing biological effects.
- If the energy contained in **1 teaspoon** of water with temperature of **60°C** would be transformed to radiation – it would **kill** a grown up man!
- **Less than 1 microgram of Po-210** can kill a man! - more toxic than any known poison (Litvinenko case)

**DNA is a Primary Target**

- Radioisotopes in DNA are more lethal than when in RNA or protein.
- Microbeam experiments show cell nucleus to be more sensitive than cytoplasm.
Why heavy ions?

Fluorescent foci marking DSB in cell nuclei

Gamma-ray irradiation:

Fe ion irradiation:

10 µm  Magnification  x 1600

Goodhead, 2013 NSRSS
Dependence RBE on LET

**LET** – Linear Energy Transfer [keV/µm]; **RBE** – Relative Biological Effectiveness

Endpoint = level of cell survival

\[ RBE = \frac{\text{Ratio of doses for identical level of biological effect (endpoint)}}{\text{endpoint}} \]
Heavy ions tracks

(Cucinotta & Durante, Lancet Oncol 2006)
Double-strand breaks (DSB) in DNA
Damage and repair kinetics of DNA after irradiation

Average number of foci per cell vs. Time after irradiation

- 1 Gy
- γ-rays
- Boron ions
Radiation Genetics

Radiation-Induced Genomic instability

- IR-exposure can cause a persistent state of instability amongst surviving cells
- Late outcomes: delayed cell death, mutator phenotypes, non-clonal aberrations – observed in the progenies of irradiated cells

Ways to measure genomic instability:

- **Delayed reproductive death**
- **Karyotypic heterogeneity**
- **Changes in mutation rates at specific loci**
- **and others**
Formation of chromosome aberrations after irradiation

Studying cytogenetic effects of low-dose γ-irradiation in human cells

- **Stable aberration**
  - Symmetrical (balanced) gene rearrangements
  - Breakage into two separate chromatids
  - Translocation: Not lethal but may release an oncogene

- **Unstable aberration**
  - Asymmetrical
  - Fragment usually lost
  - Dicentric and fragment: Usually lethal

Graphs showing the formation of aberrations per 100 cells with respect to dose in different cell types.

- Mammary carcinoma cells
- Human lymphocyte

Mathematical expressions and data points indicating the dose-response relationship for different irradiation conditions.
mFISH – multicolor Fluorescent in situ hybridization

Translocations:
Chromosomes 3 and X
Chromosomes 7 and 21
Chromosomes 7, 12 and 15
Mutagenesis – V79, HPRT gene

\[ \gamma^{(60}\text{Co)}; \text{LET} \sim 0.23 \text{ keV/\(\mu\text{m}\)*} \]

- Mutant fraction x10^4
- Expression time, days

- Data points for different doses:
  - 0.5 Gy
  - 1 Gy
  - 2 Gy
  - 3 Gy
  - 5 Gy
  - 7 Gy

- Black dashed line: Spontaneous mutants
Mutagenesis – V79, HPRT gene

$^{11}$B; LET ~ 50 keV/μm

- 0.5 Gy
- 1 Gy
- 2 Gy

Mutant fraction x10^4

Expression time, days

Spontaneous mutants
Mutagenesis – V79, HPRT gene

$^{18}$O; LET ~ 116 keV/μm

- Diamond: 0.5 Gy
- Triangle: 2 Gy

Spontaneous mutants

Mutant fraction x10$^4$

Expression time, days
Mutagenesis – V79, HPRT gene

$^{20}\text{Ne}; \text{LET} \sim 138 \text{ keV/μm}$

- 0.5 Gy
- 1 Gy
- 2 Gy
- Spontaneous mutants

Expression time, days
Mutant fraction x$10^4$
Mutagenesis – V79, HPRT gene

\[ ^{20}\text{Ne}; \text{LET} \sim 153 \text{ keV/\mu m} \]

Expression time, days

Mutant fraction \( \times 10^4 \)

- 0.5 Gy
- 1 Gy
- 2 Gy

Spontaneous mutants

20 Ne; LET ~ 153 keV/μm
$y = 2.540e^{0.013x}$

$R^2 = 0.902$
Morphological changes

Control C
Comet assay method of DNA damage detection

- D = 0 Gy
- D = 5 Gy
- D = 10 Gy
- D = 20 Gy
- D = 40 Gy
- D = 60 Gy
Space radiation

- **Galactic Cosmic Rays (GCR)** – high-energy protons and heavy ions
- **Solar Particle Events** – mainly low and medium-energy protons and electrons
  - Highly variable energy spectra
  - Rare “hard spectrum” events produce elevated fluxes up to ~ 1 GeV.
  - Main problem: **currently unpredictable**
- **Trapped Radiation** – in Low Earth Orbit
  - Van Allen Belts – trapped low energy protons and electrons

Zeitlinn, 2013 NSRSS
Why heavy ions are so important for space radiobiology?
The heavy ions of GCR (galactic cosmic rays) are the crucial factor of radiation risk for the astronauts during long-time interplanetary flights.

- The composition of GCR (~87% are protons, ~11% - helium, 1-2% - heavy ions). However, the contribution of heavy ions to the total equivalent dose of astronauts in the deep space is up to 60%.
- GCR particles can have extremely high energy and LET and is very difficult to shield an astronaut from them.
- Shielding has excessive costs and will not eliminate galactic cosmic rays (+ secondary radiation produced in shielding)
- Unique damage to biomolecules, cells, and tissues occurs from HZE ions that is qualitatively distinct from the radiation on Earth
- Exceptionally hard to simulate the GCR here on Earth (extremely low dose-rates; mixture of heavy ions etc.)
- No human data to estimate risk from heavy ions

Estimation of the dose for the Mars space travel (round-trip; no time on the surface) from the Curiosity mission:
- Current technology, shortest round-trip: 0.66 ± 0.12 Sv
  - over the NASA limit = under these conditions no astronaut can fly to Mars

Zeitlin et al., Science, 340, 2013
Galactic Cosmic Rays (GCR) in free space using the empirical Badhwar-O’Neill Model prediction for near solar minimum – for each species, integrate over energy.
Galactic Cosmic Radiation (GCR)

Abundances weighted by $Z^2$. 

Zeitlin, 2013 NSRSS
Abundances weighted by $Z^2 \times <Q>$.

Zeitlin, 2013 NSRSS
CNS Risks from Galactic Cosmic Rays (GCR)

- **Retinal flashes** observed by astronauts (suggests single heavy nuclei can disrupt brain function).
  - Central nervous system (CNS) damage by x-rays is not observed except at very high doses
- In-flight cognitive changes and late effects similar to Alzheimer’s disease are a concern for GCR.
- Cognitive tests in rats/mice show detriments at **doses as low as 10 mGy** (1 rad)
  - Studies have quantified rate of neuronal degeneration, oxidative stress, apoptosis, inflammation, and changes in dopamine function related to late CNS risks
- Large hurdle remains to establish significance in humans

**Mars mission**
- 2 – 13% of cells would be hit by at least one Fe ion during a Mars mission.
- 8 – 46% of cells would be hit by at least one particle with Z≥15 during a Mars mission.
- Every cell nucleus would be traversed by a proton once every 3

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Reduction in number of neurons (neurodegeneration) for increasing Iron doses in mouse hippocampus (J. Fike, UCSF)

Oxidative Stress (Lipid peroxidation:4-Hydroxynonenal) is Increased in Mouse Hippocampus 9 Months After 2 Gy of $^{56}$Fe Irradiation

Cucinotta, 2013 NSRSS
Cognitive test (Morris test)

$^{56}$Fe ions, 1 GeV/nucleon

Control

1.5 Gy

1 month after irradiation

Search time, s

- RADIATED
- CONTROL

Tries
Rat 214-126
Morris Water Maze
Learning Test #1

Tracking with:
Noldus Ethovision

(c) Jean-Etienne Poirrier, 2006
Cyclotron Research Center
University of Liege

jepoirrier@ulg.ac.be
http://www.poirrier.be/~jean-etienne/
Barnes maze test
Impairment of spatial cognitive functions after exposure to $^{56}$Fe

$0.2 \text{ Gy}$

$^{56}$Fe, $1 \text{ GeV/n}$

$\Phi \approx 10^5 \text{/cm}^2$

3 months after irradiation

Delay before escape, s

Day of the test
First experiments with monkeys

Irradiation with a proton medical beam, 170 MeV

Irradiation with \(^{12}\text{C}\) ions, 500 MeV/u, at the Nuclotron
Spatial perception in relation with Parkinson's disease

With Parkinson's disease, the animals make more erroneous movements.

After proton irradiation (3 Gy), significant deviations from the norm were not detected.
Irradiation with 1 Gy of 500 MeV/u carbon ions

Radiation-induced decrease in the level of neurotransmitters is observed in the brain regions responsible for the emotional and motivational state.

3 months after irradiation

- Prefrontal cortex
- Striatum
- Hippocampus
- Nucleus accumbens
- Hypothalamus

**Graphs:**
- Bar graph showing concentration (% of control) of neurotransmitters (NA) in the Nucleus accumbens.
- Line graphs showing concentration (nmol/g of tissue) of various neurotransmitters in the Hippocampus (Control vs. Irradiation).

**Note:** The graphs illustrate the changes in neurotransmitter levels following irradiation, highlighting significant reductions in certain areas of the brain.
Mathematical models of key radiation-induced DNA damage repair systems in bacterial cells were developed.

- Comprehensive computational study of radiation induced mutagenesis was performed.
- Detailed model of radiation-induced DNA double-strand break repair in mammalian and human cells was developed.


- Geant4-DNA toolkit was applied for the simulation of energy deposition processes in charged particle tracks and water radiation chemistry.

- The estimation of spatial energy and dose distributions, and the yield of radiolytic species was obtained within a single neuron and in a small neural network.


- Comprehensive models of intracellular signal transport phenomena were developed.

- The influence of low energy radiation on signals in molecular systems was studied.

In collaboration between the Space Research Institute (RAS) and FLNP (JINR), a *special facility has been constructed* at the LRB that can *model planetary soil* and allows testing prototypes of active neutron and gamma spectrometers.

- The Dynamic Albedo of Neutrons (DAN) instrument is currently working on the Mars surface on board of NASA's Curiosity rover *in cooperation with Space Research Institute RAS*.
- Helped to find water on the Moon and Mars.
Theme: "Research on Cosmic Matter on the Earth and in Nearby Space; Research on the Biological and Geochemical Specifics of the Early Earth"

Main fields of activity:

- Biogeochemical studies of cosmic dust.
- Studies of biofossils and organic compounds in meteorites and ancient terrestrial rocks.
- Studies of cosmic matter with nuclear physics methods.
Outlook for radiobiological studies at JINR

- Radiobiology and radiation genetics.
- Studying the effects of heavy ion irradiation on the structures and functions of the central nervous system.
- Neurophysiology.
- Mathematical modeling of the radiation damage of the central nervous system.
- Ground-based experiments for space radiobiology.
- Action of heavy charged particles on eye structures: the lens and retina.
- Astrobiology.
Thank you for your attention!

pavel.blahax@gmail.com