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A review of the biological effects of the low-radiation background at deep underground laboratories

Symposium on Science at PAUL

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Review

Experimental Setups for In Vitro Studies on Radon Exposure in Mammalian Cells—A Critical Overview

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Abstract: Naturally occurring radon and its short lived progeny are the second leading cause of lung cancer after smoking, and the main risk factor for non-smokers. The radon progeny, mainly Polonium-218 (^{218}Po) and Polonium-214 (^{214}Po), are responsible for the highest dose deposition in the bronchial epithelium via alpha-decay. These alpha-particles release a large amount of energy over a short penetration range, which results in severe and complex DNA damage. In order to unravel the underlying biological mechanisms which are triggered by this complex DNA damage and eventually give rise to carcinogenesis, in vitro radiobiology experiments on mammalian cells have been performed using radon exposure setups, or radon analogues, which mimic alpha-particle exposure. This review provides an overview of the different experimental setups, which have been developed and used over the past decades for in vitro radon experiments. In order to guarantee reliable results, the design and dosimetry of these setups require careful consideration, which will be emphasized in this work. Results of these in vitro experiments, particularly on bronchial epithelial cells, can provide valuable information on biomarkers, which can assist to identify exposures, as well as to study the effects of localized high dose depositions and the heterogeneous dose distribution of radon.

Keywords: radon exposure; molecular mechanisms; radon chamber; radon analogue; radiobiology; in vitro experiments; alpha particles; DNA damage

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DNA Double Strand Break Repair and Monte Carlo Dose Simulations and Measurements

by
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Co-supervisor: Dr. C. Vandevoorde and Dr O. Belov

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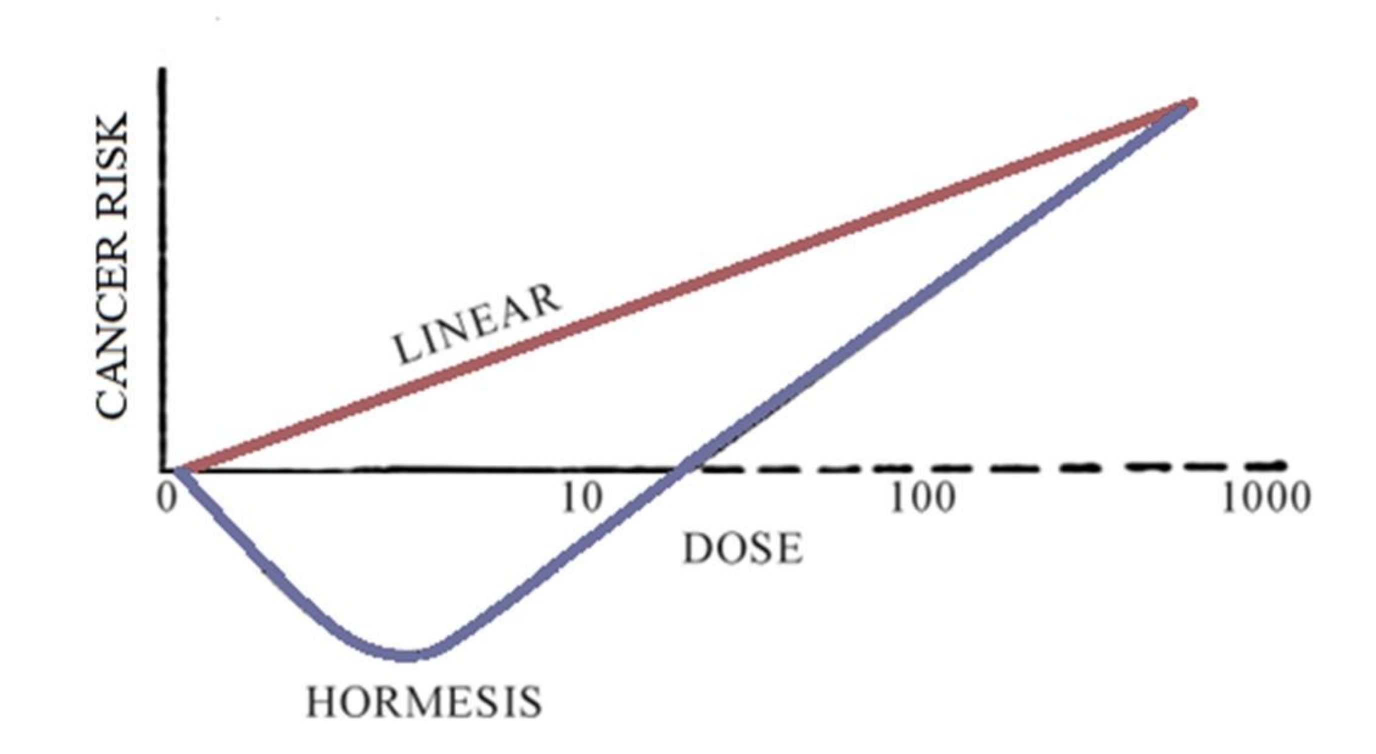
LNT vs. Hormesis



- For ~4 billion years, life has evolved in low-level ionizing radiation.
- Radiation levels in underground labs ~10 – 100 times less than standard background radiation
- What are the effects of low-levels of ionizing radiation on life?
- What can we take from atomic bomb survivor data?
- Hormesis: biphasic dose response, supralinear, sublinear, threshold

LNT vs. Hormesis

- Cells adapt in response to low doses of radiation by epigenetic gene regulation: Spontaneous changes in gene regulation that depend on the physical and chemical properties of the internal and external environments e.g. decreased antioxidant production

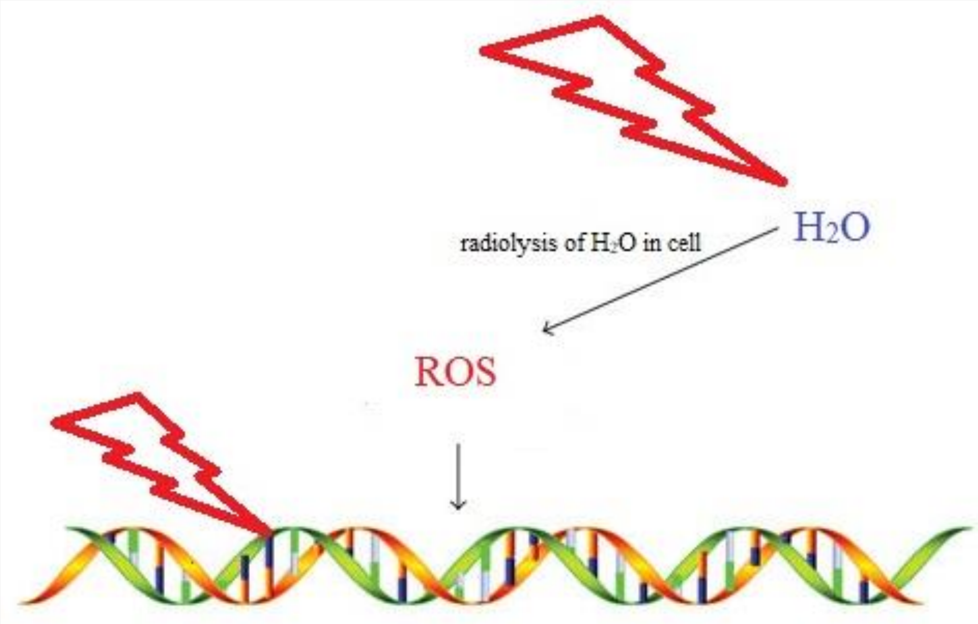
- Cells show more mutations than controls

- Standard background radiation results in antioxidant production

- Less damage from free radicals produced in the radiolysis of H_2O molecules in the cell

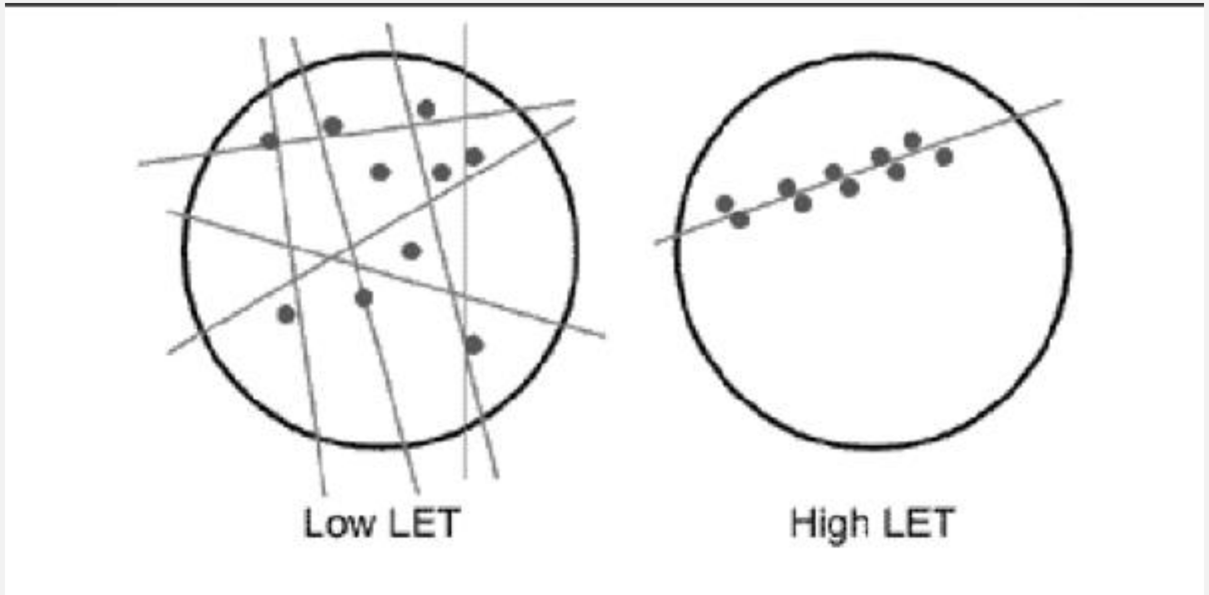
Oxidative Stress

- ROS can damage DNA indirectly
- The cell uses Superoxide dismutase (SOD) to defend against ROS
- ROS can exacerbate diseases and interact with DNA, changing the structure, causing DNA lesions, inducing single- and double-strand breaks.
- Mutations and genomic instability



The stress response

- Low LET radiation results in ROS in cells
- Created stochastically for low doses



- Frequency of ROS related to radiation dose
- Change in dose could be related to dramatic radical creation and
- Time underground decreases antioxidant production in cells
- When cells cannot scavenge free radicals, ROS can damage DNA or cause changes in gene expression
- Experiments test for proteins associated with oxidative damage and stress

Low background effects on cells

- Rodent and human cells grown for a few months at LNGS showed
- Reduced antioxidant activity
- Accumulate more radiation-induced mutations
- Decreased ability to scavenge reactive oxygen species

Radiobiological Research at Underground Labs

LNGS, SNOLAB, LSM, WIPP

What happens to organisms grown in sub-background radiation environments?

Investigating:

- Growth and development
- DNA repair capacity
- Markers of genomic damage
- Oxidative stress

Answer:

- Organisms have demonstrated a reduced ability to repair radiation and chemical induced genetic damage.
- Reduced ability to scavenge free radicals
- Growth?

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Cellular transformation assay

- Assess neoplastic transformation of CGL1 (human) cells when exposed to ionizing radiation
- Non-tumorigenic, can be transformed radiologically or chemically
- Used in vitro
- Chromogen Western Blue (WB) yields a coloured precipitant once neoplastically transformed
- Essentially foci scoring on a budget
- Sensitive to low-dose radiation exposure
- Assay period is 21 days
- Scoring works on viable or paraformaldehyde-fixed cells
- Result: increased intestinal alkaline phosphatase (ALP) = neoplastic transformation, genomic instability

At the organism level

Sreetharan S et al., 2015

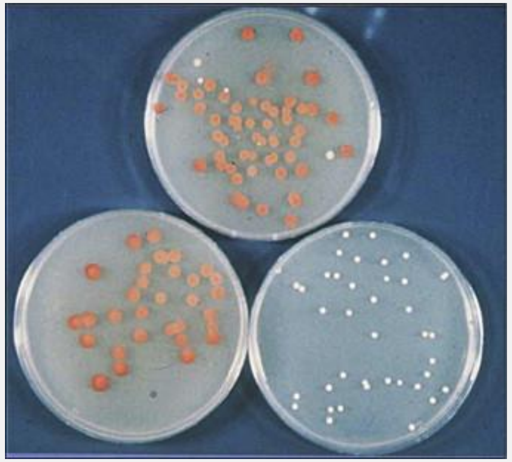
- Experiments were previously conducted on the embryos of lake whitefish at doses above background
  - Growth stimulated at 0,06 mGy/day above background
  - Growth suppressed at 2-8 Gy/day above background
- At SNOLAB the embryonic development of lake whitefish was analyzed as they are easy to raise, and were fixed at specific time points
  - Embryos were morphometrically analyzed
  - Embryos raised underground were significantly larger and weighed more than control samples
- Specialised Tissue Culture Incubator to eliminate Radon and gamma radiation

At the organism level

www.chaosofdelight.org, Nematodes

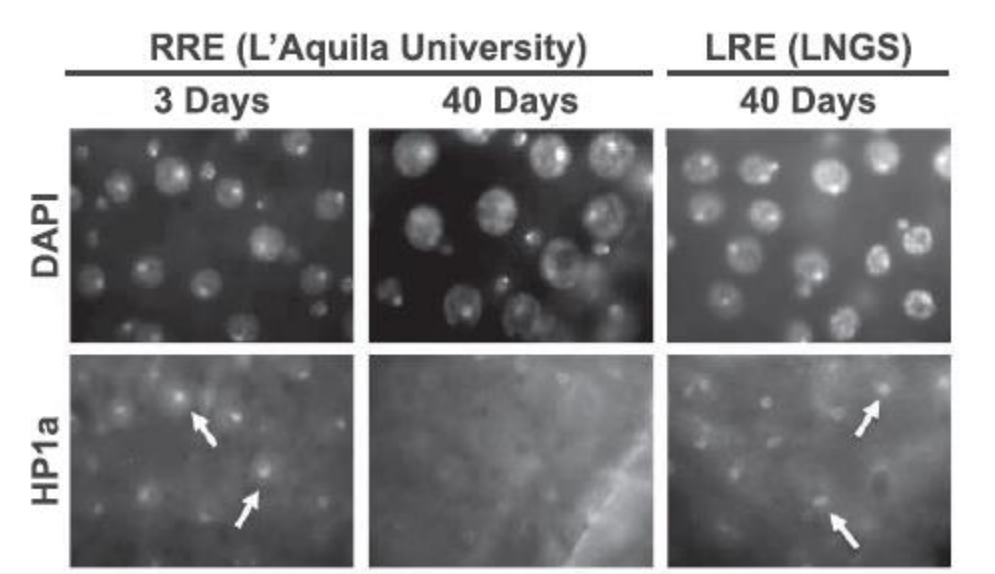
- Nematodes (Caenorhabditis elegans) – neurological development and processes SNOLAB
  - Radiation response: apoptosis, cell cycle arrest, DNA damage repair pathways, behavioural changes, memory tasks
- Yeast (Saccharomyces cerevisiae) – survival and metabolic activity SNOLAB, LNGS
  - Reduced survival and growth rates underground
  - Decreased resistance to DNA damage by chemical agents
  - Next stage:
    - investigate DNA repair capacity and cellular metabolism in the absence of NBR
    - reduce background radiation by 100x by supplementing growth medium with potassium-39

(G.G. Stewart, 2014)

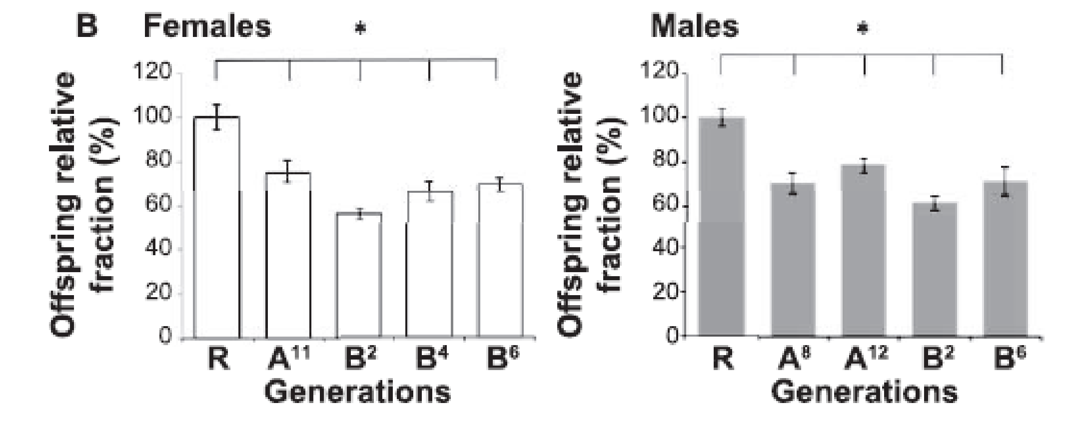


At the organism level

- Fruit flies (Drosophila melanogaster) LNGS (INFN, ISS, Rome Uni, Centro Fermi)
    - Changes in fertility, life span and motility activity (metabolic changes)
    - Median life span increased (10-15 days, several generations at low radiation) due to less muscular degeneration
    - Limited reproductive capacity in males and females by 30% after 2 generations and later
    - Spermatogenesis and oogenesis could be influenced by lower radiation background



Morciano, P. et al, 2018



At the organism level

- Fruit flies (Drosophila melanogaster) SNOLAB
  - Incubated eggs for 14 days, record hatch numbers, weight, triglyceride
  - Next stage: include additional timepoints and biological endpoints.
  - Viability affected for several generations even after moved back to standard background

Observed Effects		
Beneficial	**Harmful**	**Reference**
Cells change in response to low doses of radiation by epigenetic gene regulation (spontaneous changes in gene regulation that depend on the physical and chemical properties of the internal and external environments)		Thome et al. 2017
This can lead to a new, adapted phenotype	Enhance disease progression	
	Reduced oxidative resistance	Lampe et al. 2017, Cabone et al. 2009, Satta et al. 2002, Smith, Grof, Navarrette, & Guilmette 2011; Castillo et al. 2015
	Reduced capacity to repair DNA damage	Carbone et al. 2009
	Genomic instability	Pirkkanen et al. 2023
Embryos-increased growth		Pirkkanen et al. 2023
	Fertility decreased	Morciano, P et. al, 2018
Increased median lifespan		Morciano, P et. al, 2018

PAUL Possibilities

Control external factors that could cause cell responses:
Pressure and humidity differences
Control the lighting to maintain circadian rhythm

CGL1 transformation assay

Training biology/radiobiology students

Summer school + projects

Cryogenic freezing

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