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Summary of the PhD thesis

Contributions to the study of living organisms response to X -ray and electrons after exposure to low doses of radiation

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SUMMARY

Starting with the first observations of Pierre Curie and others regarding the radiation injury in their own bodies, the radiobiology is known as the science of biological effects of radiation and has been developed even more as radiations have been more and more used in medicine. Due to the overlapping of increased practical importance of radiations and of the incomplete understanding of the causal relationships, low dose radiation effects represent a popular subject of debate, while it is not easy to talk about radiation protection in a rational way in the conditions of still limited knowledge in the field.

The PhD thesis entitled ***" Contributions to the study of living organisms response to X -ray and electrons after exposure to low doses of radiation"*** includes experimental results on the effects of exposure to low doses of ionizing radiation (X photons and electrons) on several classes of organisms (plant, animal, bacteria).

The objective of this thesis is to highlight the responses of different types of biological materials to the exposure to ionizing radiation, either electromagnetic or corpuscular, administered in small doses – since radiobiology studies were intensified in recent years, with focus on the influence of environmental emissions of nuclear reactors during serious accidents.

The thesis consists of two parts, a first part which includes **Chapter I** and presents both a number of bibliographic references on the current state of research in the field of exposure to low doses of ionizing radiation and theoretical notions on how radiation interacts with matter and what are the biological effects induced by absorption of energy emitted by ionizing radiation.

Part two presents the personal contributions to the study of the effects of low doses of ionizing radiation and includes Chapters II - V of the thesis and at the end, Chapter VI presents the main conclusions resulted from the acquisition, processing and interpretation of experimental data.

Knowing the actual physical parameters characterizing a beam of ionizing radiation that are used in various domains: industrial, medical or research represents an important issue for the control and efficient utilization of radiation. The main field where it is necessary to perform dosimetric measurements in order to determine the parameters defining the quality of the radiation beams and to check up their consistency with the existing standards is the radiotherapy, because quality assurance is extremely important both for patient and for occupationally exposed workers, reducing the likelihood of occurrence of accidents and errors.

Chapter II presents the results of the quality control tests for the X-rays and electron beams performance that have been used in the experimental studies contained in this manuscript. All the tests were conducted in the Radiotherapy and Oncology Department of the Emergency County Hospital "St. Spiridon", for a Varian 2100SC clinical linear particle accelerator. This device has several treatment options with multiple energies: photons (the voltage of 6 MV and 10 MV) and electrons (with energies of 6 MeV, 9 MeV, 12 MeV and 15 MeV).

From the dosimetric measurements parameters such as: percentage of depth dose distribution on the central axis of the beam, the dose distribution outside the central axis of the beam of radiation and dose profile, were determined to ensure the accuracy of absorbed dose estimation in the exposed biological samples.

The irradiation time required to deliver the applied radiation doses was calculated for a source to sample distance (SSD) of 100 cm, using the following formula:

$$\mathbf{t = D/\dot{D}\,(z_{max},10,h\nu)\times RDF(A,h\nu)\times PDD(1{,}5cm,A,h\nu)\times 0{,}005029}$$

where ***t*** is the irradiation time, ***D*** is the prescribed dose for each sample, $\boldsymbol{\dot{D}}$ *(*z_{max}*)* is the dose rate at the point (at depth z) of the maximum dose along the central axis of a 10x10cm^2 radiation field size, ***RDF*** (A, hν) is the relative dose factor, ***PDD*** (1.5 cm, A, hν**)** is the percentage depth dose for *A* radiation field size. The values for *D, RDF* and *PDD* for the given irradiation geometry (photon or electron beams) were obtained from calibration procedures (standard dosimetry IAEA TRS-398) using a standard 3D Blue Water Phantom, a Freiburg PTW Markus flat ionizing chamber – for the electron beam, a PTW Farmer cylindrical ionization chamber- for the photon beam and a PTW UNIDOS electrometer.

In **Chapter III**, spectral and cytogenetic analysis methods were used to highlight the effects of low-dose radiation exposure on plant organisms in the early stages of their development, aiming the study of the induced effects in the synthesis processes of important substances involved in the development of plants and the induced effects at the cellular level by analyzing the changes of the mitotic division index and the induction of cytogenetic aberrations at the cellular level.

All experiments on biological effects of low doses of ionizing radiation action on vegetable organisms were made on the same biological material - *Zea mays*, (the maize), a plant species with economic importance. The X-ray exposure was achieved by

single irradiation dose with 0.5Gy, 0.75Gy, 1Gy, 2Gy and 2.5Gy respectively.

Following the cytogenetic investigations for over 30,000 cells, the main types of chromosomal aberrations that could be identified were single or multiple bridges, ana-telophases with delayed or expelled chromosomes and multipolar divisions.

Exposure to low doses of X-rays caused an increase in mitotic cell division for 0.5Gy and 0.75Gy, with a maximum of 9.15% corresponding to samples exposed to a dose of 0.75Gy - which can suggest a stimulating effect on the mitotic activity after exposure to low doses.

The increased mitotic index for this range of doses was due to the accumulation of cells in prophase, the frequency of cells in other phases of cell division varied less compared to the control. It was found that the decrease in the mitotic index and the mitotic division delay are dose dependent. Also the cytotoxicity of low-dose X-rays on the studied plant embryos was demonstrated, because the percentage of chromosomal aberrations increased up to ten times compared to the nom irradiated samples.

At the end of Chapter III, the conclusions that were drawn from processing the data from the experiments conducted in order to identify the biological effects induced by exposure to low doses of ionizing radiation (X photons and electrons) into the vegetable organisms during the early stages of their development are presented:

1. The pigment concentrations (chlorophyll a, chlorophyll b, carotenoids) were slightly affected by exposure to X-rays, as we recorded their synthesis inhibition with increasing the dose. Thus, for the 2Gy radiation dose the chlorophyll *a* concentration decreased from 0.742 mg/g of tissue at 0.624 mg/g of tissue, value comparable with that of the control samples. The reduction can be

attributed to the harmful effect of oxidative stress induced by free radicals produced by ionizing radiation as such pigments could be consumed when participating in eliminating free radicals and dissipation of energy. Slow decrease can be explained by the fact that the antioxidant defense system counterbalances destabilizing effects at the studied range of doses.

2. After cytogenetic analysis it was found that mitotic index varies considerably with the irradiation dose. Thus, at low doses (0.5 to 0.75Gy) there is a mitotic excess while for higher doses (1, 2, 2.5Gy) the mitotic index decreases because some cells are impeded to enter the mitosis at the right time. Irradiation essentially acts as synchronizer agents by selective affecting of the cells which are found in the above mentioned phases and determine the delay in their progression to mitosis.

3. Experimental data resulting from the studies on the influence of water content of the seeds indicated the following:

– The presoaking of the *Zea mays* seeds in distillated water determinates a low but steady decline with statistically significance of the mitotic index, compared to the control, in the samples exposed to low doses of either X-rays or accelerated electrons, complementary an increased percentage of cells at mitotic rest occurring;

– Comparing the total number of aberrations induced in two experimental variants that differ in the water content, it can be seen that the X-ray exposure was able to double the cytotoxic effect at doses of 0.5Gy and 6Gy respectively in the hydrated samples; electron beam exposure of the hydrated samples led to progressively increase of the cytotoxic effect unlike in dry samples;

– A slight stimulating effect of biosynthesis process of the nucleic acids occurs in plants grown from dry seeds up to 3Gy

followed by an inhibition of the synthesis of DNA and RNA, while for hydrated seeds, there is a decrease in the nucleic acids content by 18% compared to non-irradiated samples;

– In the case of the plants grown from dry seeds exposed to 3Gy electron beam, the same trend for boosting the biosynthesis of nucleic acids as for photon beam exposure was observed, by up to 17% compared with the control (RNA, $p < 0.05$); after 0.5Gy electron beam exposure of presoaked seed the DNA biosynthesis decreased by 30% compared to the control.

Motivated by the aim to obtain information about the possibility of side effects of bacterial origin in patients who are receiving radiation therapy, and taking into account the possible presence of *Staphylococcus aureus* germs on the skin or in the irradiated internal organs in **Chapter IV**, entitled ***"Experimental investigations on pathogen germs response to low doses of ionizing radiation exposure***" we presented the experimental results that highlight the side effects of radiotherapy mediated by the microbial load of irradiated organs. The influence of irradiation with doses comparable to those used in radiotherapy (X-rays and electrons) on the resistance of some microorganisms to antibiotics and the cultures cell multiplication rate changes were studied.

Regarding the inhibitory effect of X radiation exposure on the *S. aureus* germs, the experimental data showed that the relative density of cells (irradiated samples cell density / non-irradiated samples cell density) of studied bacterial cultures progressively decreased, by 35 and 75% after exposure to 31Gy and 60Gy doses, corresponding to irradiation times of 25 min. and 50 min respectively. Cell density increased progressively in irradiated samples for longer periods of time, for 65, 85 and 100 min,

corresponding to doses of 87, 108, 128Gy, keeping still lower than in control samples, as a result of physiological adaptation to radiation.

The influence of radiation exposure on antibiotic resistance was evaluated by measuring the inhibition zones of microbial growth diameters on agar medium for ampicillin (A), chloramphenicol (C), tetracycline (T), tobramycin (TOB) and ofloxacin (OF) and comparing them with control non-irradiated samples. Thus the samples irradiated for 24 min. showed an increase of the inhibition zone diameter compared to the control against all 5 tested antibiotics. The inhibition zones diameter resulting from the antibiotic diffusion was generally increased with up to 15% for bacterial samples X-ray exposed with 31 and 128Gy which correspond to irradiation times between 25 and 100 min. The growth of the inhibition zone diameter suggested a slight decrease in antibiotic resistance of the studied *S. aureus* cultures.

To evaluate the effectiveness of sterilization after electron beam exposure the number of colonies forming units was determined for each sample. Thus, low dose (31Gy - irradiation time of 16 min.) was found to stimulate the process of multiplication of bacteria by up to 12.5% compared to control samples. The exposure to higher doses resulted in a slower multiplication process, leading to the formation of only 7 colonies (5 times less than the control samples) in 68 min. irradiated samples (128Gy).

For the X-rays exposure, the plot in logarithmic coordinates suggests a sigmoid survival curve with an inflection point. The evidenced cell density decreasing in all irradiated samples compared to non-irradiated ones was statistically significant (applying the *t*-test resulted $p < 0.05$).

It is well known that the harmful effects of ionizing radiation exposure of the biological systems are mainly mediated by generation of reactive oxygen species in cells as a result of water radiolysis [2-9]. Oxidative stress may contribute to radio-induced cytotoxicity and metabolic as well as morphological changes in organisms subjected to radiotherapy, in animal or vegetable organisms subjected to various accidental irradiation exposures or controlled irradiation exposures such as outer space experiments [10]. Starting from this presumption, in **Chapter V** are included experimental results obtained from the studies regarding the influence of low doses of ionizing radiation on certain types of animal tissues (by catalase activity measurements) and the blood cells (by spectrophotometric estimation of hemolysis intensity). The hemolytic effects were not noticeable for irradiation with low doses ranging from 0.25 to 0.5Gy in both the X-ray beam and the electron beam exposure, compared with effects induced by exposure to medium doses (1-6Gy).

Catalase is an antioxidant enzyme ubiquitously present in mammalian and non-mammalian aerobic cells containing a cytochrome system. It was initially isolated from ox liver and later from blood, bacterial, and plant sources. The enzyme contains 4 ferrihemoprotein groups per molecule. Catalase activity varies greatly for different tissues. The activity is highest in the liver and kidney, and lowest in connective tissues. Catalase catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in the eukaryotic cell as a by-product of various oxidase and superoxide dismutase reactions. Hydrogen peroxide is highly deleterious to the cell and its accumulation causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death [11-12]. Removal of the H_2O_2 from the cell by catalase provides protection against

oxidative damage to the cell. Against oxidative stress, cells are equipped with enzymatic and non-enzymatic antioxidant defence systems. A major defence mechanism involves the antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT) which convert active oxygen molecules into non-toxic compounds. The liver has the highest contents of antioxidants and antioxidant enzymes indicating that it plays an important role in pro-oxidants detoxification [13].

After processing the experimental data, a significant increase in catalase activity in the irradiated liver tissue samples has been observed, compared to the non-irradiated ones. An X- ray exposure to a low dose of 0.5Gy caused an increase in hepatic antioxidant defense process by about 2.3 times compared with non-irradiated samples ($p<0.05$). Exposure to 2Gy resulted in tripling the intensity of catalase activity compared to non-irradiated samples. The same significant increase was found in the case of electron beam exposure, determining the intensification of liver antioxidant defense almost 3 times compared with non-irradiated liver tissue samples.

In conclusion, the obtained experimental data indicated an increase in enzymatic activity of catalase in the studied various types of tissues. Thus, exposure to 0.5Gy resulted in an increase in catalase activity with percentages between 20% and 25% in samples of brain, muscle and lung. Because the liver and kidney antioxidant enzymes content is higher, the catalase enzyme activity was found to be increased by 25% in kidney and up to 2.3 times in the liver samples compared to non-irradiated tissue samples. A 2Gy tissue exposure caused a greater increase in enzyme activity (up to 3 times compared to the control in the case of liver tissue), both after X-ray and electron beam exposure.

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SCIENTIFIC ACTIVITY

I. PAPERS PUBLISHED IN ISI JOURNALS

1. *"Bacteria response to non-thermal physical factors:* A study on *Staphylococcus aureus*", Focea Ramona, Poiata Antoniea, Motrescu Iuliana, Nastuta Andrei, Creanga Dorina and Popa Gheorghe, **African Journal of Biotechnology**, vol.11(18):423-4240, march 2012.

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II. PAPERS PUBLISHED IN IDB / B+ / B (CNCSIS)

1. *"Low dose X-ray effects on catalase activity in animal tissue",* Ramona FOCEA, Claudia NADEJDE, Dorina CREANGA, Tudor LUCHIAN, **Journal of Physics: Conference Series (JPCS)**, submitted.

2. *Experimental investigations on plant organisms response to the action of ionizing radiations",* Ramona Focea, Dorina Creanga , ***Bule. Institutului Politehnic din Iasi, (Secţia Matematică, Mecanică teoretică, Fizică),*** Tomul LVI(LX), Fasc.4, Editura POLITEHNIUM, 2010, ISBN ISSN 1244-7863, pp. 267-273.

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Between October 2009 and September 2012 I attended to 10 national and international conferences.