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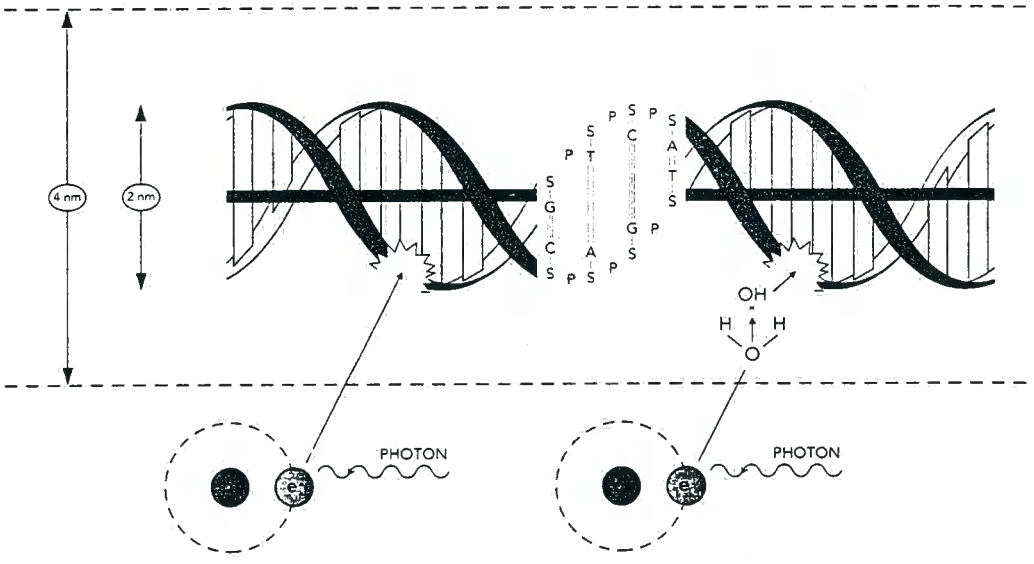
STUDIECENTRUM VOOR KERNENERGIE
CENTRE D'ÉTUDE DE L'ÉNERGIE NUCLÉAIRE

Topical Day on Biological Effects of Radiation



SCK•CEN
May 15, 1997

BLG-739

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Editors

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Seminar Location

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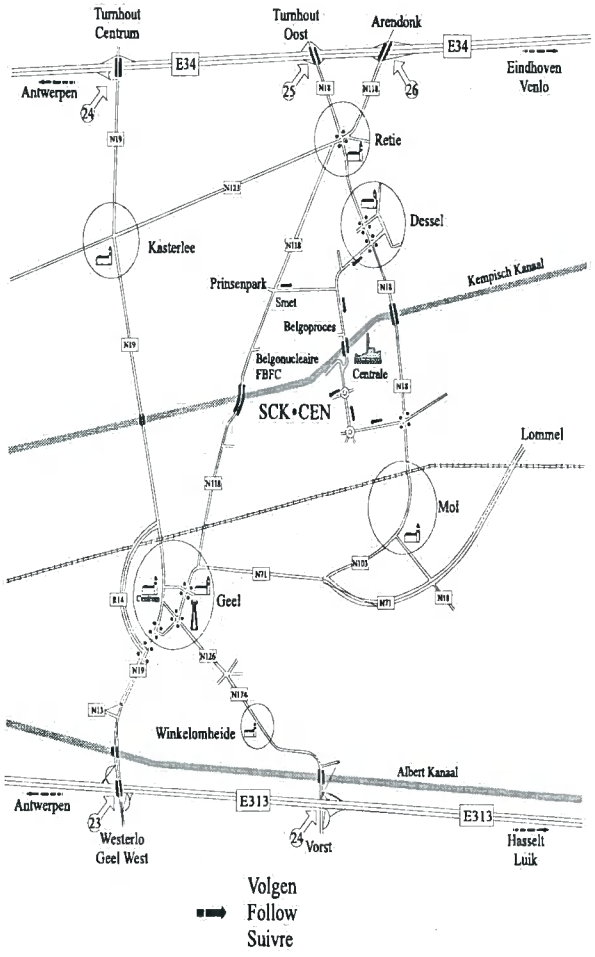

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Announcement and Registration

Topical Day on Biological Effects of Radiation

SCK•CEN, Mol
Conference Hall VITO
May 15, 1997

SCK•CEN
B-2400 Mol
Belgium

The SCK•CEN

The SCK•CEN (Belgian Nuclear Research Centre) was created in 1952. The mission of SCK•CEN is focused on nuclear science and technology, in particular: nuclear safety and reactor experiments; radioactive waste management and dismantling of nuclear facilities; radiation protection and health physics. The SCK•CEN has built in more than 40 years an international reputation in these disciplines.

Aim

The aim of this topical day is to give an information on the potential effects of ionizing radiation on human health. A first lecture will give a general overview on molecular and biophysical aspects of radiation, its effects on cells and organisms, and the contribution of radiobiology to radiation protection and risk assessment. Other lectures will be devoted to more specific topics such as the genetic effects of radiation and its effects on the developing organism, the results of epidemiological studies in man, the effects of radiation on the cell cycle and the mechanisms of radiation-induced apoptosis.

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Programme

09.00 Welcoming word
Paul Govaerts, General Manager

09.10 Radiological protection and radiobiology: the link
Felix Luykx (President of the Department Advisory Committee on Radiation Protection and Site Restoration)

09.30 Radiobiology, a support for radioprotection
Georg Gerber (Department Advisory Committee on Radiation Protection and Site Restoration)

10.10 Coffee break

10.30 Recent developments in radiation genetics
Alain Léonard (Unité de Tératogenèse et Mutagenèse, UCL)

11.10 Effects of radiation on the developing organism
Paul Jacquet

11.50 Health effects of ionizing radiation: epidemiological data in men
Hilde Engels

12.30 Lunch offered by the SCK•CEN

14.00 Cell cycle checkpoints and irradiation response
Thomas Jung (Institute of Radiation Hygiene, German Federal Office for Radiation Protection, Munich)

14.40 Radiation-induced apoptosis in the thymus
Marie-Paule Defresne (Département d'Anatomie et Cytologie pathologiques, Université de Liège)

15.20 Coffee break

15.40 Oocyte/embryo fusion as a tool in MPF and cell cycle research
Josef Fulka, Jr. (Institute of Animal Production, Prague)

16.10 Effects of radiation on cell cycle regulation in the early mammalian embryo
Sarah Baatout

16.40 Health effects of ionizing radiation: the multidisciplinary approach of Radiobiology
Lucile Baugnet-Mahieu

17.00 Closing remarks

17.15 Drink

Registration

If you want to attend this Topical Day, please fill in this form and send it back before May 8, 1997.
(Please write in capital letters)

☐ Yes, I will attend the Topical Day on Biological Effects of Radiation on May 15, 1997.

I will participate to: *(Please tick as appropriate)*

Morning session	☐ yes	☐ no
Lunch	☐ yes	☐ no
I request vegetarian food	☐ yes	☐ no
Afternoon session	☐ yes	☐ no
Drink	☐ yes	☐ no

Name:

First Name:

☐ Prof ☐ Dr ☐ Ir ☐ Mr ☐ Ms

Inst., Company, Org.:

Department:

Position:

Mailing address:

Tel.:

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Topical Day on Biological Effects of Radiation

SCK•CEN
May 15, 1997

BLG-739

Contents

Radiological protection - Radiobiology : The link.

Felix Luykx
Department Advisory Committee on Radiation Protection and Site Restoration

The primary aim of radiation protection is to provide an appropriate standard of protection for man against the harmful effects of ionizing radiation without unduly limiting the beneficial practices giving rise to radiation exposure.

The International Commission on Radiological Protection (ICRP) has always tried to base its protection approach on the best available information on the biological effects of ionizing radiation and has used this information to establish a simplified, but adequate basis for radiological protection. Over the years there has been, however, an evolution in the development of protection recommendations, which has lead to the current system. This system of radiological protection is shortly described.

The basic framework as formulated now by ICRP is :
- to prevent the occurrence of deterministic effects, by keeping the doses below the relevant thresholds and
- to ensure that all reasonable steps are taken to reduce the induction of stochastic effects.

Since there is little evidence of harm at levels of annual dose which are near or below the dose limits recommended by ICRP, a good deal of scientific judgement is required in predicting the probability of harm resulting from low doses.

What radiological protection expects from radiobiological research is to refine the evaluation of harm at low doses and low dose rates, by exploring the mechanisms by which ionizing radiation acts on living tissue.

Radiobiology, a support for radioprotection.

Georg Gerber
Department Advisory Committee on Radiation Protection and Site Restoration

Outline of Lecture

- Molecular and biophysical aspects of radiatio
- Effects on cells;
- Effects on organism:
  - stochastic effects (cancer and genetic damage),
  - deterministic effects,
  - effects on the developing organism;
- Contributions of radiation biology to radiation protection and risk assessment.

Aspects of the Molecular Action of Radiation

- Energy deposited in matter through ionisations/excitations can bring about a variety of chemical transformations, some directly damaging the biological molecule, some doing so via reactive intermediates (direct/indirect action);
- The type of radiation determines the spatial distribution of energy absorption events: if they are closely spaced (clustered damage from high LET radiation), damage to a target is more serious and less well repairable than that from low LET radiation;
- Radiation effects can be modified in the presence of other agents: oxygen and radiosensitizers increase, radioprotectors (eg radical scavengers) decrease radiation damage;
- The most important target for radiation in the cell is DNA because of its uniqueness, importance and size;
- Enzymatic repair of DNA can restore the original structure but can also lead to mis-repair resulting in biological damage.

Doses for Deterministic Damage

Tissue	Acute Dose Sv	Chronic Dose Sv	Appearance Time
Bone marrow			
Minimal treatment	3.5	~7	5-15 days
Support. treatment	5	~10	
Intensive treatment	10	~20	
Gastrointest. Tract	15	45?	1-7 days
Lung	10	~30	weeks +
Skin			
Erythema	6	10	2 weeks
Deep injury	20	35+	months +
Thyroid	10	50	weeks +
Cataract	3	6	months +

Information on Radiation Consequences and Risks

comes from

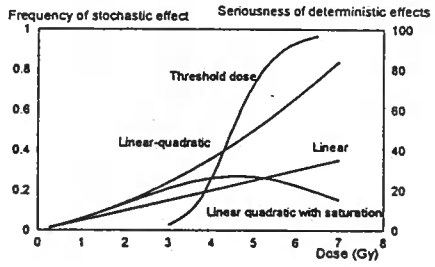
- Biophysical models relating the events of energy absorption (microdosimetry) to the biological structure and effect;
- Definition of molecular damage ,especially in DNA, and its subsequent repair;
- Studies on isolated cell systems dealing with clonal death, malignant transformation, mutations and the influence of repair deficiencies;
- Animal experiments using different radiation modalities and radionuclides;
- Clinical observations on radiotherapy patients and persons exposed accidentally;
- Epidemiology of populations exposed from atomic bombs, from medical procedures or during work.

Action of Radiation on Cells II

Genetic Aspects

- **Chromosome aberrations:** Severe (major deletions, important translocations leading to dicentrics, rings...) with significant loss of genetic information result in reproductive death, less severe ones (eg balanced translocations) can cause mutations in somatic or germ cells;
- **Mutations:** Changes in function and structure of cells, often compatible with cell survival, if induced in germ cells, can be transmitted to progeny;
- **Malignant transformation:** Due to genetic damage (activation of oncogenes....) if followed by promotion, progression and proliferation of the affected cell can produce clinical tumour disease.

Dose-effect Relationships



Action of Radiation on Cells I

Cell survival

- **Reproductive death:** Cells rendered incapable of forming a clone, perish after one or a few divisions due to severe genetic damage. Do is ~ 0.5 -2 Gy, extrapolation number 1-10, the ratio a/b ~ 0.5-1 Gy;
- **Effects on cell cycle:**
  - arrest in G2 phase (~1 h /Gy) when permanent, can sometimes lead to the formation of giant cells,
  - arrest in G1 phase: dividing cells can be delayed or kept from entering DNA synthesis (S phase) and possibly be diverted towards apoptosis; non-dividing (Go) cells can become unable to divide when stimulated;
- **Apoptosis:** Death before cells divide (interphase death) in certain cells (eg lymphocytes) after ~ 1 Gy, in others after higher doses.

Types of Consequences of Radiation Exposure

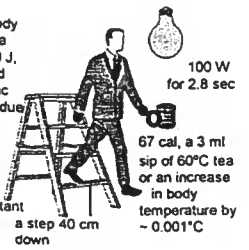
- **Stochastic:** arise without a threshold with a frequency dependent on dose but a severity independent of dose; they result from damage to a single or few cells. They are relevant for protection of workers and the population from low doses/low dose rates.
  - cancer can develop in the exposed person from 5 - 10 years later,
  - hereditary damage can appear in the progeny of the exposed person.
- **Deterministic:** arise only above a threshold with a severity dependent on dose; they result from damage to a substantial percentage of cells (eg death if >99.9% of bone marrow stem cells are damaged) and are relevant for situations where dose limits have been substantially exceeded.
  - acute radiation syndromes within hours to months (bone marrow, intestine skin etc) with local or general symptoms,
  - chronic radiation syndromes after months to years, often from damage to blood vessels, fibrosis and/or permanent cell loss, mostly localized.
- **Developing organism:** probably with a very small (<0.5 Gy) threshold, relevant for the protection of pregnant women.

Mechanisms of Deterministic Damage

- Reproductive death in the stem cell compartments of cell replacement systems causes loss of functional cells with concomitant symptoms arising after a delay dependent on cell replacement parameters (5 d for intestine, 1-2 w for blood, skin, > 50 d for lung, testis...) and following doses which are high enough to destroy a sufficiently large proportion of stem cells.
- Damage to slowly or not replacing cells (brain) causes effects due to cell loss weeks and months later (atrophy).
- Damage to blood vessels (endothelial cells) causes late atrophy,
- Fibrosis can result from an activation and differentiation of fibroblasts subsequent to cell loss or somatic mutation.

Characteristics of Ionizing Radiation II

The dose (4 Gy) of whole body X-ray exposure that can kill a man (70 kg) amounts to 280 J, a small amount, if expressed as kinetic, electrical or caloric energy. This discrepancy is due to the fact that ionizing radiation deposits large amounts of energy in an inhomogeneous manner on an atomic level thereby damaging biologically important molecules.



Detriment from Genetic Damage

- Very serious genetic damage (loss of large amounts of genetic material) resulting in abortion, often at a time when pregnancy is still unknown, or in neonatal death, causes no, or limited, suffering to the individual and no harm to society;
- Serious genetic damage to first generation (dominant mutations, malformations, trisomies) resulting in early death or considerable long-term functional impairment and suffering, sometimes requiring the lifelong stay in institutions; can have serious individual and societal consequences.
- Genetic damage to later generations (recessive mutations) contribute to the genetic load of humanity and can cause variable harm to the individual and society,
- Multi-factorial diseases (allergies, constitutional diseases) can result in a reduction in quality of life and societal costs.

Radiobiology Contributes to Radiation Protection, Medical Applications of Radiation and General Biology by Helping to

- elucidate the molecular biology of DNA repair, modifications of cell cycle and cell death;
- explain the mechanisms by which radiation-induced and other cancers arise;
- understand the risks from low doses/low dose rates for which neither human nor animal data are available on the basis of biophysical models, molecular biology and animal experiments in context with epidemiological data;
- assess genetic risks in animals and extrapolate the data to man;
- provide information on metabolism and risk of radionuclides for which no human information is available;
- improve radiotherapy modalities for malignant diseases;

Recent development in radiation genetics.

Alain Léonard
Teratogenicity and Mutagenicity Unit
Catholic University of Louvain

This presentation will not restrict itself to a review of recent advanced in the evaluation of hereditary effects resulting from exposure to ionizing radiation but rather will attempt to answer the following three crucial questions:

1) Does the exposure situation, considered on an international scale, still justify to devote substantial funds to the study of hereditary effects?
2) Is the information presently available sufficient to provide a reliable assessment of such risks?
3) Do we still have the economic means and scientific manpower to perform any further studies needed in this area?

1) Regarding the first question it should be recalled that we are still burdened, to some extent, by the unfortunate legacy of the many atmospheric atomic tests carried out in the past without adequate protection. In addition, radioactivity continues to enter the environment due to inadequate stockage or disposal of radioactive waste. Finally, one can never entirely exclude the possibility of an accident in nuclear plants or an atomic war.

2) None of the epidemiological studies on the survivors of the atomic bombs in Hiroshima and Nagasaki, on people exposed in the Chernobyl accident or those living in areas of high natural radioactivity have revealed a statistically significant increase in hereditary effects in man. On the basis of experimental results in laboratory animals, the doubling dose or indirect method which excludes multifactorial disorders estimates the risk at 3 mutations per 1000 persons per Sievert for the next 2 generations, whereas the direct method which considers only clinically important disorder expressed in the first generation arrives at a risk of 2-4 mutations par 1000 persons per Sievert.

3) Most laboratories specialized in classical radiation genetics are disappearing, few funds are made available, and the classical methods of mammalian genetics presently available do not promise spectacular advances in the quantitative assessment of hereditary risks.

In conclusion, I do not consider it appropriate at the present time to continue the study of hereditary risks of radiation unless by way of new approaches to the problem. Since we know already that such effects can occur, even we cannot detect them, we should concentrate on urgent measures to combat the radioactive contamination of the environment.

Effects of radiation on the developing organism.

Paul Jacquet
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Nuclear Reactor Safety and Radiation Protection
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The embryonic development can be subdivided into three periods: (1) the preimplantation period, which extends from fertilization to the time when the embryo attaches to the wall of the uterus; (2) the organogenesis period, during which the major organs are developed; and (3) the foetal period, during which growth of the newly formed organs takes place. Experiments performed in laboratory animals have shown that each of these periods is characterized by a particular sensitivity to ionizing radiation or other toxic agents. Thus, it is widely believed that an irradiation during the preimplantation period may essentially result in the death of the embryo. Irradiation during the phase of organogenesis may characteristically result in the production of a variety of congenital anomalies. The induction of anomalies depends on the number of damaged cells in the forming organ and will not occur below a certain threshold (100 mGy in the mouse). Irradiation during the foetal period can induce anomalies in the development of the tissues and general or localized growth retardation. The growth retardation induced during this period frequently persists during all the extra-uterine life.
In man, radiation-induced malformations of body structures other than the central nervous system are uncommon. However, exposure of the human conceptus during the last part of pregnancy, the foetal period, may lead to severe mental retardation associated or not with microcephaly (decrease in head diameter). Data obtained in children who had been exposed *in utero* to the A-bombs in Hiroshima and Nagasaki showed that the probability of SMR (severe mental retardation) is essentially zero for an exposure occurring at less than 8 weeks of gestational age, is maximal for an exposure during the period 8-15 weeks (95 % lower limit of the threshold : 0.06-0.31 Gy) and decreases during the period 16-25 weeks (95 % lower limit of the threshold: 0.28 Gy) (the estimates of the thresholds are those of Otake and colleagues published in December 1996).
In addition to these effects, some studies have established a link between irradiation *in utero* and the subsequent development of leukaemia and other childhood malignancies. Although far from being proved, this risk is now accepted by the most important scientific committees on account of prudence (2.78 %/Gy).
Overall, for small doses, the detriment calculated from the risk estimates for the different potential effects on the developing organism appears rather small, in comparison with the natural risks, spontaneously associated with pregnancy.

Health effects of ionizing radiation: epidemiological data in men.

Hilde Engels
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Most of the epidemiological studies about health effects of ionizing radiation in men try to estimate cancer risk in populations with short-term exposure to high doses of radiation. The most important groups that have been studied are the atomic bomb survivors of Hiroshima and Nagasaki (Life Span Study), patients that have been irradiated for diagnostic or therapeutic purposes, occupationally exposed workers such as radium dial painters, radiologists or uranium miners, and populations exposed to radioactive fall out. So far, cancer risks of long-term exposure to low doses of ionizing radiation have mainly been estimated through extrapolation of the data from the Life Span Study. Current radiation protection recommendations are generally based on these estimates. The extrapolations are subject to uncertainties, and therefore direct assessment of the carcinogenic effects in populations with long-term low-level radiation exposure is important. A number of epidemiological studies are on-going in populations living in areas with high background radiation and among workers of the nuclear industry. No specific cancer type however has been associated with ionizing radiation so far, and many of these epidemiological studies lack statistical power to identify small variations in baseline cancer incidence. A large multicenter study has recently been set up by the International Agency for Research on Cancer (IARC/WHO) in Lyon, France, to study cancer risk in nuclear workers: data from 14 countries will be combined to obtain more precise risk estimates. Both the high dose studies and the first combined analysis of nuclear workers studies will be reviewed in this presentation.

Cell cycle checkpoints and irradiation response.

Thomas Jung
Institute of Radiation Hygiene
German Federal Office for Radiation Protection
Munich, Germany

Passage of cells through the cell cycle requires the complete and error-free copying of the entire genome, as well as the accurate segregation of sister chromatids to daughter cells. At several transition points the cell cycle is subject to controls. Some of these reflect the action of extracellular growth factors and serve to integrate cell proliferation with cell growth and differentiation. Others, termed checkpoint controls, originate from within the cell and arise from the need to coordinate different cell cycle events in space and time, and to halt cell cycle progression in response to irregularities such as DNA damage. Ionizing radiation as well as UV radiation induce DNA-damage and subsequently lead to the activation of cell cycle checkpoints and cell cycle blocks.

A family of protein kinases, termed cyclin-dependent kinases (CDKs), controls the transition between successive phases of the cell cycle in all eukaryotic cells. All CDKs are structurally related to each other, and all require associated cyclin proteins for activity.

Multiple phosphorylation and dephosphorylation events occur in both CDK and cyclin subunits. In the case of p34cdk1 phosphorylation of threonine 14 and tyrosine 15, to neighboring residues with in the ATP binding site, causes the cdk1/cyclin B complexes to be inactive until the G2/M transition, when a dual specific phosphatase (cdc25) removes the phosphates and causes activation of the kinases. Ionizing radiation induces phosphorylation of p34cdk1 and subsequently inactivation of the cdk1/cyclin B complex and therefore a cell cycle block in G2.

Inhibitors of CDKs and CDK/cyclin complexes control CDK activity in multiple ways. Cells with damaged DNA arrest their cell cycle at three stages: in G1, in S and in G2. G1 and S phase arrest in response to DNA damage is mediated via wild-type p53. Cells with dominant negative mutation of p53 no longer arrest at G1 and S on damage. Damage-induced p53 is transcriptionally active and induces transcription of p21, an inhibitor of CDKs. p21 complexes with cyclin, CDK and PCNA. It inhibits kinase activity of CDKs, mainly of cdk2/cyclin E and cdk2/cyclin A complexes. This leads to cell cycle arrests in G1 and S.

The cardinal role of CDK/cyclin complexes in cell cycle regulation is now well established. The effects of ionizing radiation are critically dependent on the amount of radiation-induced primary DNA damage which is transformed into heritable damage (mutation). DNA repair and cell cycle arrests, i.e. the extra time a cell requires for repair, are the key elements in this transformation process.

Radiation-induced apoptosis in the thymus.

Marie-Paul Defresne
Department of pathologic Anatomy and Cytology
University of Liège

The subject of cell death has recently become a focus of interest for researchers from a variety of diverse fields. In preparation for and during cell death, a complex cascade of biological events, typical of cell life, takes place. These processes involve activation of many regulatory pathways, preservation and often modulation of transcriptional and translational activities, activation of many diverse enzyme systems, modification of the cell plasma membrane structure and function, etc... The changes which accompany death are irreversible and culminate with cessation of biological activity.

It has been generally accepted that apoptosis and necrosis are two distinct, mutually exclusive modes of cell death. Apoptosis is an active and physiological mode of cell death in which the cell itself designs and executes the program of its own demise and subsequent body disposal. A multistep mechanism regulates the cell's propensity to respond to various stimuli by apoptosis, whose complexity has only recently become apparent. The regulation system involves the presence of at least two distinct checkpoints, one controlled by the bcl-2/bax family of proteins, another cy caspases. Through several oncogenes and tumor suppressor genes such as p53, this system interacts with the machinery regulating cell proliferation and DNA repair.

However, all the regulatory elements involved in apoptosis are not yet known. To elucidate the cascade of events culminating in apoptosis, some organs such as the thymus are very useful: indeed, the thymus is very sensitive to apoptosis induction by a wide range of external stimuli, among which irradiation. The present work describes the kinetic of thymic apoptosis after irradiation in relation with the space and time differential expression of genes known to be involved in the regulation of apoptosis as well as that of a new putative regulator of apoptosis, called c-cbl. We show that p53 and cbl seem to be involved in radiation-induced apoptosis while it is less evident for bax. There is strong evidence that bcl-2 plays a role in the radio-resistance of some cells, which permits subsequent thymic regeneration. The function of Cbl protein in the process of apoptosis is now under investigation.

Oocyte/embryo fusion as a tool in MPF and cell cycle research.

J. Fulka, Jr.
Institute of Animal Production
Prague
Czech Republic

The induced cell-to-fusion is a very useful approach which has a wide range application in:

i/ cell biology studies
ii/ cell cycle studies
iii/ radiation experiments
iiii/ production of cloned animals

Fusion can be induced in different ways (electrofusion, polyethyleneglycol, Sendai virus - induced fusion) and, if we calculate that only two cells are fused, the following combinations can be constructed:

a/ fusion of two interphase cells
b/ one interphase cell is fused to one M-phase cell (mitotic, meiotic)
c/ two M-phase cells (meiotic, mitotic) are fused

The potential application of this method (fusion) and results we can obtain and expect in the above mentioned experiments and combinations are discussed in detail in our contribution.

Effects of radiation on cell cycle regulation in the early mammalian embryo.

Sarah Baatout
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The Maturation Promoting Factor (MPF) periodically becomes active during the course of meiotic maturation and the mitotic cell cycle and is universally recognized as the biological entity responsible for driving the cell cycle from the G2- to M-phase.
During meiosis, MPF activity appears shortly before germinal vesicle breakdown and fluctuates thereafter in accord with the cell cycle; activity is high at metaphase I and II and low during anaphase and telophase.
MPF is a protein kinase, of which p34cdc2 is the catalytic subunit and cyclin B, the regulatory subunit. Cyclin accumulates during S and G2 phases and becomes degraded at the metaphase/anaphase transition. It associates with p34cdc2, and the formation of this complex allows for the phosphorylation of p34cdc2 at several sites maintaining it in an inactive state and ensuring that mitosis does not occur prematurely. In animal cells, MPF is activated by the dephosphorylation of tyr-15 and thr-14 residues.
A number of proteins are phosphorylated by protein kinases when cells enter M-phase, including the histone H1 which is the most used biochemical indicator of the biological MPF activity in mammalian cells.
Irradiation results in delayed progression through G1, S or G2 phases of the cell cycle. Although this has been known for many years, the mechanisms leading to the so-called G2-arrest are not very well understood. Our studies focus on the mechanisms responsible for the G2-delay in the early mouse embryos following irradiation.

Young female BALB/c mice were induced to superovulate by intraperitoneal injection of 5 i.u. pregnant mare serum (PMS) followed 45-48 h later by 5 i.u. human chorionic gonadotrophin (hCG). Superovulation occurs at 12 ± 3 h after hCG injection. In order to dispose of highly synchronous embryonic populations, females were individually caged with males of the same strain from 15 to 17 h after hCG injection. Fertilization of the positive females was considered to have occurred at the middle of this short mating period. One-cell embryos were collected 8 h after presumed fertilization and cultured *in vitro* for different times. Following a method described by Jung, oocytes and embryos were analyzed for MPF activity using : histone H1 as the exogenous substrate, ^{32}P-ATP to allow the phosphorylation of the substrate and protein kinase inhibitor peptide to block cyclic AMP-dependent protein kinase. Histones were separated on 1D-SDS electrophoresis gels in Mini Protean II cell. After autoradiography of the gels, the histone H1 kinase activity of MPF was analyzed by liquid scintillation counting after excision of the bands corresponding to the histone H1. The detection threshold allowed to measure the histone H1 kinase activity of single oocytes or one-cell embryos. Unspecific non-enzymatic labeling of histone H1 was measured by performing the complete kinase reaction without oocytes.

The MPF activity was high in ovulated oocytes in metaphase II (activity referred to as 100%) and low in embryos at the early pronuclear stage (8.1% ± 1.2). Similar differences were found between one-cell embryos blocked in the metaphase of the first mitotic

division by colchicine (100 %) and early 2-cell embryos (25.9 % ± 1.3). The MPF activity was also measured in oocytes in metaphase II at different times after ovulation and was shown to significantly decrease 54 hours after ovulation. We also compared 1- and 2-cell embryos in mitosis and found that their MPF activity was similar.
In a following set of experiments, MPF activity was compared between control one-cell embryos and others X-irradiated with 2.5 Gy at the early pronuclear stage. Such a treatment is known to induce a G2-delay of about 20 hours in at least 80% of the embryos. In the X-irradiated embryos, the histone H1 kinase activity remained at a low level during the whole period of G2-arrest. In irradiated embryos escaping the G2-arrest, the MPF activity during the first mitosis was slightly lower than in control embryos dividing at the same time. In irradiated embryos dividing after G2-arrest, the MPF activity was still very low and only slightly higher than in those that remained blocked in G2. Irradiated embryos at the 2-cell stage showed a small G2-delay and the MPF activity in those after the G2-delay was similar to control 2-cell embryos in mitosis.

There seems to be a relationship between the levels of MPF activity in mitosis and the health status of the embryo :

- most embryos escaping G2-arrest after 2.5 Gy given at the one-cell stage will die after a reduced number of cell divisions (4 or 5). Those embryos showed already reduced levels of activity at their first division.
- one-cell embryos able to divide after G2-arrest will die soon thereafter, at the 2- or 4-cell stage. Those embryos showed extremely low levels of MPF activity at this late first division.

The action of radiation on MPF activity in one-cell embryos could result from two distinct mechanisms : a prevention of activation of the complex by radiation or an effect on its stability. Immunoblotting studies will allow to recognize changes in the state of phosphorylation of specific MPF subunits in function of the state of G2 arrest in one-cell embryos and are currently under progress. The effects of radiation on the stability of the MPF complex will require the fusion experiments between oocytes and one-cell embryos.

Health effects of ionizing radiation : the multidisciplinary approach of radiobiology.

Lucile Baugnet-Mahieu
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Radiobiological research is based on fundamental disciplines: physics, chemistry, molecular biology, but the applications of radiobiology are clearly linked to the public health: radiation protection, oncology, radiotherapy and nuclear medicine.

With regard to the radiation protection, radiobiological research aims to improve the evaluation of the potential risks of low doses, delivered at low dose rates, by exploring the mechanisms of action of ionizing radiation at the molecular and cellular levels and their consequences to the organisms.
Since the discovery of the double helix, the DNA has been considered as the main target for the ionizing radiation: the radiation-induced DNA lesions, their consequences at the cellular and tissue levels and the biochemical mechanisms of DNA repair have been studied extensively. Recently, the spectacular advances and the dramatic improvements of the techniques in molecular genetics allowed to identify some of the genes involved in the early and late response to ionizing radiation.
The contribution of radiobiological research to radiation protection and risk assessment has been nicely demonstrated by all the distinguished speakers of this topical day.

The close relationship between radiobiology and oncology may be illustrated by the recent publication in Cell (january 1997) of a paper entitled:
"Association of BRCA1 with RAD51 in mitotic and meiotic cells"
- BRCA1 is the first gene identified in the familial cases of breast and ovarian cancers.
- RAD51 is a gene playing an important role in the repair of DNA damages.

In normal individuals, both oncosuppressor genes are involved in the control of cell cycle progression and the maintenance of genomic integrity. By using not only cancer cell lines but also human spermatocytes, the authors of the study demonstrate a functional interaction between a cancer gene and a gene involved in the radiosensitivity, in both mitotic and meiotic cell cycle control.

Concerning the connection between the radiobiology and the medical applications of ionizing radiation and radionuclides, a series of topics, out of the subjects treated today, are most important from the radiobiological point of vue.
For instance:
- the potential risks of the radiation doses originating from X-ray and nuclear medicine procedures;
- the radiobiological bases of modern treatment modalities in teletherapy and brachytherapy;
- the use of radionuclides and radiopharmaceuticals for tumor targeting;
- the palliative effects of ^{89}Sr or phosphonates labelled with beta emitters such as ^{186}Re or ^{153}Sm, in case of bone metastases, etc...

Different radioisotopes for radiotherapy or nuclear medicine have been produced for years at the reactors of the CEN•SCK, but at the present time, we are particularly concerned about the production of ^{99}Molybdenum, the parent isotope of ^{99m}Technetium. Indeed, more than 70% of the radiopharmaceuticals used for the diagnostics are labelled with Technetium, due to the unique physico-chemical properties and the relatively low cost of the isotope. Only a few companies in the world are able to provide the hospitals with Technetium generators and the number of reactors used as neutron sources to irradiate ^{235}Uranium targets is decreasing in developed countries. Fortunately, the BR-2 reactor started again in april 1997 after refurbishment and is able to provide the IRE with irradiated Uranium. An interesting alternative, studied at the CEN•SCK in collaboration with Ion Beam Applications, is the project ADONIS (project leader: Luc Van Den Durpel). The neutrons, needed to irradiate the Uranium target, should be obtained by coupling a cyclotron with a subcritical fuel assembly.

Conclusions:

One of the missions of the Belgian Nuclear Study Center is to maintain the scientific know-how needed:

- to advise the authorities on radiation protection issues concerning potential hazards from exposure to ionizing radiation;
- to provide the general public with adequate information on topics related to the health effects of ionizing radiation.

The advisory committee for radiation protection and a panel of external experts recommended for the section radiobiology:

- to maintain research programmes focused on the radiosensitivity of developing mammalian organisms;
- to carry out these activities in close collaboration with the Belgian and European scientific community;
- to keep abreast of new advances, potentially relevant for the SCK•CEN, with regard to the medical.