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Damage Propagation in Complex Biological Systems Following Exposure to Low Doses of Ionizing Radiation

Ludwig E. Feinendegen[1], Herwig Paretzke[2] and Ronald D. Neumann[3]

1. Department of Nuclear Medicine, Heinrich-Heine University, Düsseldorf, Germany; and Medical Department, Brookhaven National Laboratory, Upton, NY, USA
2. Institute of Radioprotection, Research Center for Health and Environmental Sciences (GSF), Neuherberg, Munich, Germany
3. Department of Nuclear Medicine, Clinical Center, The National Institutes of Health, Bethesda, MD, USA.

Abstract : Biological organisms contain hierarchical organization, from atoms to molecules, cells, tissues, organs, and the whole organism. Complex signaling by molecules within and between cells controls homeostasis and adaptation of the whole organism against perturbations. Energy depositions from ionizing radiation in tissue micro-masses trigger stochastically local molecular events that may result in structural damage with consequent short or long term changes in function. Damage to DNA in a tissue element increases over a certain dose range linearly with the energy deposited. This is a defined risk of DNA damage production by the energy deposition events. A second risk describes the probability of damage propagation from the primary site at the basic level of molecular organization to higher levels of the organism. This second risk depends on both quality and quantity of perturbations received at the basic level, and on the resistance by homeostatic controls against transfer of such damage. The homeostatic signaling that controls the second risk at different levels does not respond to small perturbations in a linear fashion. Moreover, protective responses under homeostatic control at the various levels of biological organization may become up-regulated temporarily and specifically by low level perturbations. These adaptive responses reflect the tolerance of homeostatic control, and, thus, also depend on "dose". The low-dose induced temporary up-regulations of protection may operate also against non-radiogenic perturbations; for instance against those from metabolic products, such as reactive oxygen species (ROS). At single cell exposures below ≅ 0.1 Gy, adaptive protections against propagation of non-radiogenic damage often tend to outweigh permanent manifestations of radiogenic damage. The quality and extent of homeostatic responses are under genetic control. Thus, complex systems responses encompass both damage and prevention of further damage and its propagation to higher levels; they are expected to vary among individuals. The balance between health risk and benefit of low-level radiation exposure of any given individual may become predictable by gene-expression profiles in un-irradiated and irradiated tissue cells of this individual at some future time.

Key word : Damage Propagation, Biological Systems, Low Doses, Ionizing Radiation

Introduction

Assessment of biological effects, be they expressing risk or benefit, in radiological exposures at low level exposure is currently faced with great uncertainties that derive from the basic sciences in radiation biology and molecular biology, as well as from epidemiology. A major uncertainty lies in the high prevalence of malignant diseases especially in older populations whereas ionizing radiation is a comparatively very weak carcinogen even at higher doses, the effect of which is most difficult to quantify in necessarily limited numbers of exposed people (Mosmann, 2007). Epidemiology will hardly ever contribute to solve this uncertainty for statistical reasons. On the other hand, research in cells,

tissues, and animals over the past few decades increasingly provide evidence that low doses of ionizing radiation initiate biological responses that were unexpected and often different from previously known responses at higher exposure and exposure rate levels. In fact, entirely new phenomena have been uncovered such as low-dose induced delayed appearing, and temporarily lasting cellular signaling changes affecting intracellular enzymes activities, reactions to reactive oxygen species, DNA synthesis and repair, apoptosis, cell differentiation, and immune responses (Feinendegen *et al.*, 2007). These responses occur in conjunction with altered gene expression patterns and also express the capacity of the biological system to adaptively protect itself against renewed potentially damaging toxins. These low-dose specific cell responses are described in the context of other newly recognized phenomena which may also arise after high dose irradiation. These second category responses predominantly encompass both the so-called "bystander effects" (Mothersill and Seymour, 2006), as well as genomic instability that may befall cellular progeny over many cell generations (Kadhim *et al.*, 2006).

All these relatively new experimental findings appear to embrace a common pattern, despite the broad variation of their nature and of the biological systems in which they were observed. The plethora of data on low-dose effects clearly challenges the claim that the dose-risk function most likely adheres to the linear-no-threshold hypothesis. This hypothesis has its origin in the seminal discoveries by Müller beginning in 1927 (Müller, 1927), with the subsequent observation that radiation may cause gene mutations proportionally to the absorbed dose over the full dose range studied**.**

The present contribution continues the efforts published previously (Feinendegen *et al.*, 1995; Feinendegen *et al.*, 1999; Feinendegen *et al.*, 2000; Feinendegen *et al.*, 2004; Feinendegen *et al.*, 2005) to present a conceptual frame that allows to accommodate coherently the various data in the light of system radiation biology.

Principal components of radiation-response systems and their interactions in homeostasis

Low level exposure of biological systems to ionizing radiation nearly always affects multiple sites at the molecular level randomly in the irradiated cells and tissues. In order to understand responses to low doses the principle structure and function of the exposed system needs consideration.

The evolution of biological organisms brings increasing complexity towards structurally defined levels of organization in a hierarchical manner, as schematically seen in **Figure 1**: At the basic level are the atoms (99 % of them are C, H, O, N, S, and P). These constitute also metabolically relevant molecules that consist of 2 to some ~ 10^4 atoms. The molecules are structurally highly defined to serve both as architectural building blocks as well as metabolic functional units, and ca. 80 % of them by weight constitute water. There are about 10^{11} such molecules per individual cell each complete with a full set of genes. With their particular functions cells operate as a whole and are fundamental units of life. About 10^9 cells are on average in 1 gram of tissue and organ, each one serving in specific ways to guarantee the proper structural and functional integrity of

the tissue and organs and, thus, the entire organism. The organism operates as a whole using multiple intricate nets of signaling that provide for homeostasis at all levels of organization even in a potentially life threatening environment (Feinendegen *et al.*, 2005).

The predominant signaling tools are electrons as well as molecules that can recognize each other because of their chemical and physical properties. One may, in principle, discern three types of signaling loops within a whole body. There is the signaling between molecules within cells, between cells of a given tissue or organ, and between different tissues and/or organs. All signaling, wherever it occurs, involves cells that have receptors, usually highly specialized for uniquely interacting with, and to produce, molecular ligands as signals.

Figure 1 schematically conveys the fact that signaling between and at each hierarchical level of organization tends to keep the whole organism in a state of adaptive homeostasis, *i.e.*, at steady state of all metabolic reactions and operations in the face of unavoidable perturbations that challenge all levels of organization from both external and internal sources. If a perturbation at a given level of organization is relatively small, the system mostly returns to steady state in nearly an immediate response by intertwined feed-back controls to protect system integrity. With increasing gravity of a perturbation at a given level the organism suffers "stress", often with the consequent generation of delayed secondary reactions generally referred to as "adaptive responses". These equip the organism - usually temporarily - with an improved capacity for defending against, and reconstituting itself in the face of, a renewed "attack" of a similar quality, and for even more efficiently returning to its steady state operation. In case of severely disruptive perturbations at a low hierarchical level of organization, the local damage produces challenges for the higher levels of organism responses designed to restore an organism's homeostasis by damage removal, architectural remodeling and functional reconstruction. An example is the common experience of wound healing after a macroscopic tissue injury. Disease evolves when the protective barriers against damage propagation through ascending hierarchical levels are overwhelmed by damage and, thus, loose their capability to regain homeostasis.

Homeostatic perturbations by ionizing radiation

Ionizing radiation primarily interacts with atoms at the initiation of, and along, charged particle tracks, caused by deposition of energy along the track passage in cell-tissue microscopic regions, thus creating "event packages", according to the radiation quality (ICRU, 1983; Paretzke, 1987). Such events encompass molecular modifications on site by direct action and secondarily mainly by reactive oxygen species (ROS) from radiogenic hydrolysis (Hall, 2000). A special case worth mentioning here is that of the so-called Auger effect. This effect elicits grossly mixed types of radiation quality that arise on the one hand from multiple electron tracks and also from a charged atom. For instance, each decay of 125-iodine within the confines of the DNA molecule causes at least one severe double strand break at the molecular site of the decay from charge transfer processes and, thus, acts as a tool of "molecular surgery"; in addition, numerous other DNA damages occur aweay from the decay site in the affected cell from the

multitude of electron tracks associated with Auger effects.(Feinendegen and Neumann, 2004).

Any of these stochastically distributed events triggers biological responses by affected cells, be they hit directly, or activated indirectly by signaling through the tissue matrix from "hit" cells, *i.e.* through bystander effects (Mothersill and Seymour, 2006). With increasing total energy deposited by a given radiation quality, and, thus, with increasing numbers of initial triggering particle tracks biological responses eventually may affect a whole organ or even the whole organism at every level of its biological organization. Nevertheless, the generation of responses wherever they occur appears to always originate in cells. In other words, homeostatic perturbations may be triggered by energy deposition events anywhere in the system and with their numbers increasing may become observable to engulf successive higher organizational levels such as tissues, organs and even the whole body (Fliedner *et al.*, 2005). The probability of such an observation, obviously, largely depends on the type, quality, and extent of initial homeostatic perturbations at the molecular level, and on the tolerance of homeostatic controls operating at subsequent higher levels via signaling within and between cells, and between cells of different tissues and/or organs, all under homeostatic control (Arthur *et al*., 2000).

Principal physiological barriers against radiation-induced diseases

Normal organisms command an array of immediately operative physiological barriers that protect against immediate and late consequences of potentially life-threatening impacts. Such impacts may occur at any level of biological organization. An example of barrier at the tissue level is the skin that protects against manifold mechanical stresses and if injured, will initiate protective responses leading, for instance, to wound healing through signal-induced cell proliferation and differentiation. Operating at various ascending levels of the organism, the barriers actually form a sequence of defenses against the propagation of any exo- or endogenous damage sufficient to evolve into clinical disease, as shown schematically in **Figure 2.** Otherwise, a human organism made up by more than 10^{13} complicated cellular entities would never have a chance of living up to 100 years**.** Thus, with a holistic view of systemic function one may discern (Feinendegen *et al*., 2007; Feinendegen *et al.*, 1995; Feinendegen *et al.*, 1999; Feinendegen *et al.*, 2000; Feinendegen *et al.*, 2004; Feinendegen *et al.*, 2005) :

a) defenses via scavenging mechanisms at the atomic-molecular level;

b) molecular repair especially of DNA, with reconstitution of essential cell constituents and functions;

c) removal of damaged cells from tissue either by induced cell death, *i.e.*, apoptosis, or by stem cell differentiation, or by a stimulated immune response. Depending on the degree of disturbance repair often is associated by replacement of damaged and/or lost cells for maintenance of tissue function**.**

These immediate responses against potentially damaging events are quite well understood especially regarding DNA damage from exposure to ionizing radiation (Hall, 2000). Within minutes after irradiation

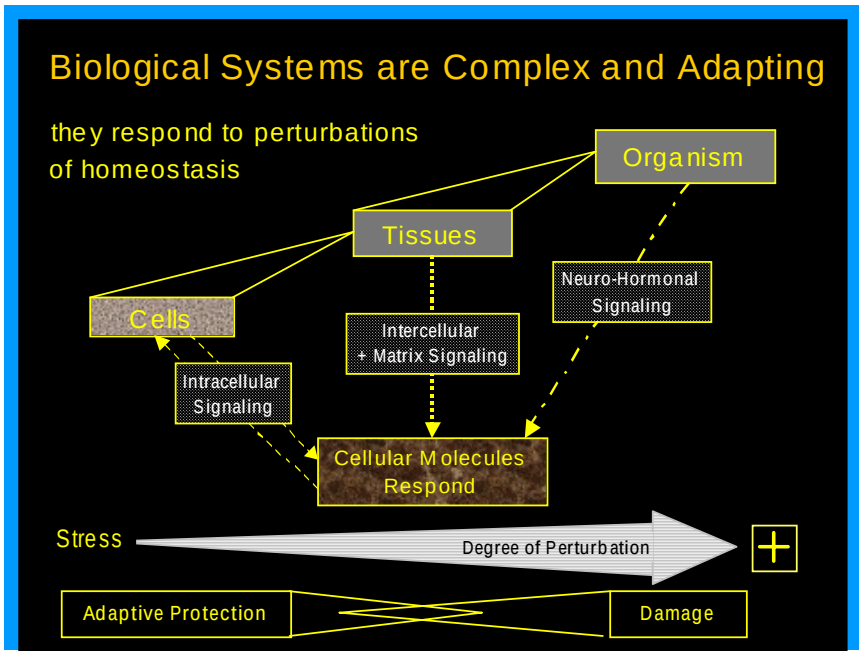
Biological Systems are Complex and Adapting
they respond to perturbations of homeostasis
Organism
Tissues
Cells
Neuro-Hormonal Signaling
Intercellular + Matrix Signaling
Intracellular Signaling
Cellular Molecules Respond
Stress
Degree of Perturbation
Adaptive Protection
Damage

Fig. 1

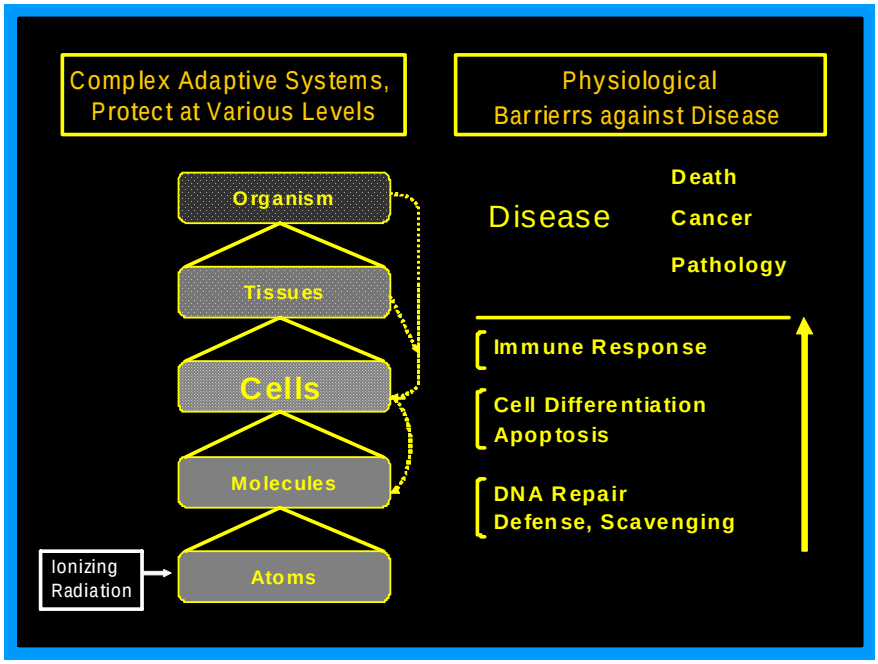
Complex Adaptive Systems, Protect at Various Levels
Physiological Barrierrs against Disease
Organism
Tissues
Cells
Molecules
Atoms
Ionizing Radiation
Disease
Death
Cancer
Pathology
Immune Response
Cell Differentiation
Apoptosis
DNA Repair
Defense, Scavenging

Fig. 2

there is a plethora of DNA modifications including a broad distribution of number and quality of DNA double strand breaks per cell, which can be visualized and counted per individual cell by immuno-histochemical methods. (Rothkamm and Löbrich,. 2003). Usually well within 24 hours, the fluorescent foci, supposedly indicative of double strand breaks, decrease to a lower number, closer to that of the background "spontaneous", *i.e.* pre-irradiation, number. The capacity of normal cells to repair DNA and other cellular damage is genetically determined in accordance with the individual's genome. Today, more than 150 genes have been described to be involved in DNA repair at high doses; other genes in a similar number are active in low-dose stress response. Each gene has the potential to vary among individuals through polymorphisms, mutations, and even deletions from the individual's genome. In general, then, initial non-lethal radiation damage is answered readily by immediate attempts at structural reconstitution with regained functional homeostasis.

One should note that the various barriers are, in a way, twofold. On the one hand, there is a preexisting physical or chemical protection preventing an impact from disrupting a biological structure with the concomitant disturbance of homeostasis. On the other hand, there is also an immediate biochemical-cellular response with rapid signaling for gene activation required for reconstitution of structure and function. Both of these types of immediate protections are known to operate not linearly with the degree of energy impact and its ensuing primary damage. In fact, immediate protective responses appear as deterministic types of responses. This means, an energy impact of a given level must be large enough to overcome a threshold of neutralization before structure and/or function are perturbed sufficiently to elicit the organism's response to restore homeostasis. Such principal deterministic response patterns are observable at any level of biological organization, even at the basic molecular level such as DNA (Bond and Varma, 1995).

There are many common daily experiences with this principle response pattern. Thus, for tissue damage to occur and trigger a biological response, an object causing a contusion, for instance, must have a certain force before cells are disrupted to cause local bleeding or an open wound which then generates local healing. Below this level of force there will be no harm from an interaction of a blunt object with tissue such as skin. Another common example relates to infectious disease. In the presence of antibodies, infectious disease develops only above a given minimal inoculation of micro-organisms that depends on the degree of toxicity of the microorganism and on the status of the body's immune system. Below this level, local cellular reactions and protective antibodies, if present, operate immediately and effectively. A third example is the extent and duration of sun-bathing that may damage DNA and cells. A certain intensity and duration of exposure is needed before the skin reacts and turns red or even into a sun burn.

In general then, only when damage causing homeostatic perturbations overwhelms structural and functional barriers at successive levels, from the molecular to cellular to tissue level, disease can develop sometimes with a potentially severe or even lethal outcome. Since increasing doses of ionizing radiation eventually paralyze barriers

at all hierarchical levels, higher radiation doses in large target volumes may allow damage at basic levels of organization to propagate with minimal or no inhibition and thus to evolve into clinically evident disease. As a consequence, many, but definitely not all, dose-response functions expectedly tend to be linear at higher doses, but not so at low doses. Because the above-mentioned physiological barriers at all levels are under genetic control, certain defects in the involved genes, which control these physiological protections, may change individual radiation sensitivity drastically (Cleaver, 1968). However, one should keep in mind that in most cases diseases are controlled by a set of at least several genes.

The physiological barriers sketched out above may also operate against the development of some types of clinical cancer. Even if the protective mechanisms against cancer are still not fully understood, their effects are obvious. An illustrative example is the relationship between the extent of DNA damage caused by radiation and the probability of cancer induction in the exposed individual. Thus, the ratio of radiation induced DNA double strand breaks including those of the multi-damage site-type in a potentially oncogenic blood-forming stem cell and of lethal leukemia has been estimated to be close to 10^{12} (Feinendegen *et al.*, 1995), or even higher; this data has its source in experimental and epidemiological observations. The claim that even a single DNA double strand break, however grave, in a stem cell may cause cancer is scientifically unjustified. Low-dose induced cancer is, nevertheless, believed by many for practical and applicable reasons to increase proportionally with dose under the assumption that a certain, however small, fraction of radiogenically transformed cells could escape barriers and expands into clinical cancer:

Adaptive protection at ascending levels of biological organization

Adaptive protections need consideration especially in those instances when homeostatic perturbation at a given level is relatively large but below the level of barrier "break-through", *i.e.* in stress situations. Adaptive responses are well known, for instance, following so-called oxygen stress (Finkel and Holbrook 2000; Chandra *et al.*, 2000). Also, low level exposure to ionizing radiation can change cellular signaling with temporary changes in enzyme activities that are involved in protecting against reactive oxygen species (ROS) and in DNA synthesis (Zamboglou *et al.*, 1981; Feinendegen *et al.*, 1984), DNA repair (Olivieri *et al.*, 1984; Wolff *et al.*, 1988), and DNA damage removal by various routes (Feinendegen *et al.*, 2007; Feinendegen *et al.*, 1995; Feinendegen *et al.*, 1999; Feinendegen *et al.*, 2000; Feinendegen *et al.*, 2004; Feinendegen *et al.*, 2005; Kondo 1988; James and Makinodan 1990; Tubiana *et al.*, 2006). These and other well-documented cell and tissue responses to low level radiation exposure are currently understood to be the consequences of delayed and mostly temporary up-regulation of physiological barriers against the propagation of damage from the lower to the higher levels of biological organization. These responses summarily here referred to as adaptive protections generally begin to operate within a few hours after toxic exposure, and may last from hours to months depending on the type of protection. It appears that adaptive protection against ROS lasts a shorter period of time than the

protective up-regulation of DNA repair, which in turn seems to last shorter than the low-dose induced stimulation of the immune system lasting at least for several months or longer, as schematically illustrated in **Figure 3** (Feinendegen *et al.*, 2007; Feinendegen *et al.*, 1995; Feinendegen *et al.*, 1999; Feinendegen *et al.*, 2000; Feinendegen *et al.*, 2004; Feinendegen *et al.*, 2005; Feinendegen, 2005).

These delayed appearing and mostly temporary up-regulations of existing barriers can be observed at very low doses in the range of mGy and show on average a maximum effectiveness at an acute dose of about 0.1 Gy. They disappear as doses increase beyond 0.2 Gy of low-LET radiation and are hardly, or not at all, seen anymore beyond about 0.5 Gy (Feinendegen *et al.*, 2007; Feinendegen, 2005; Feinendegen *et al.*, 1996). However, the probability of apoptosis, if one classifies it as an expression of adaptive protection, apparently increases linearly beyond 0.5 Gy over a certain dose region. **Figure 4** is, like **Figure 3,** a schematic presentation of average values from many available observations (for review Feinendegen *et al.*, 2007; Feinendegen *et al.*, 1995; Feinendegen *et al.*, 1999; Feinendegen *et al.*, 2000; Feinendegen *et al.*, 2004; Feinendegen *et al.*, 2005; Tubiana *et al.*, 2006).

The low-dose specific response pattern in terms of adaptive protection is in agreement with data on gene activity modulation after low- and high-dose irradiation (Tubiana *et al.*, 2006; Franco *et al.*, 2005). Low-level radiation exposure modulates the activities of a set of genes, mostly involved in stress responses. These genes do not respond to high-level exposure, and vice versa. But a large number of genes respond to both low- and high-level irradiation. Thus, in one recent study, out of 10500 genes in human keratinocytes a total of 853 genes were modulated between 3 to 74 hours after irradiation, and of these 214 only appeared changed with an irradiation by 1 cGy, mainly stress response genes. The high dose of 2 Gy modulated the activities of 639 genes. Both doses changed the expression in 269 genes. This set of data shows statistically a highly significant difference between gene activity responses at low and high doses (Franco *et al.*, 2005).

Adaptive protection, again, is a common physiological phenomenon, understood in every day's life. Body building through properly dosed training stress is a well known experience. Immunization by small amounts of microorganisms against outbreak of disease caused by larger amounts of this microorganism is again common knowledge and basis of vaccination. Examples at the cellular level are responses to increasing concentrations of ROS (Sen *et al.*, 2000).

In a normal cell about 10^9 ROS molecules arise in the cytoplasm outside mitochondria on average per day, mainly from metabolic reactions; and additional small ROS bursts come from various responses to external cell signaling (Sen *et al.*, 2000; Pollycove and Feinendegen, 2003). When an average electron, for instance produced by 100 kV x-rays, hits a cell, about 150 ROS occur in that cell within a fraction of a millisecond**.** Supra-basal bursts of ROS either from metabolic

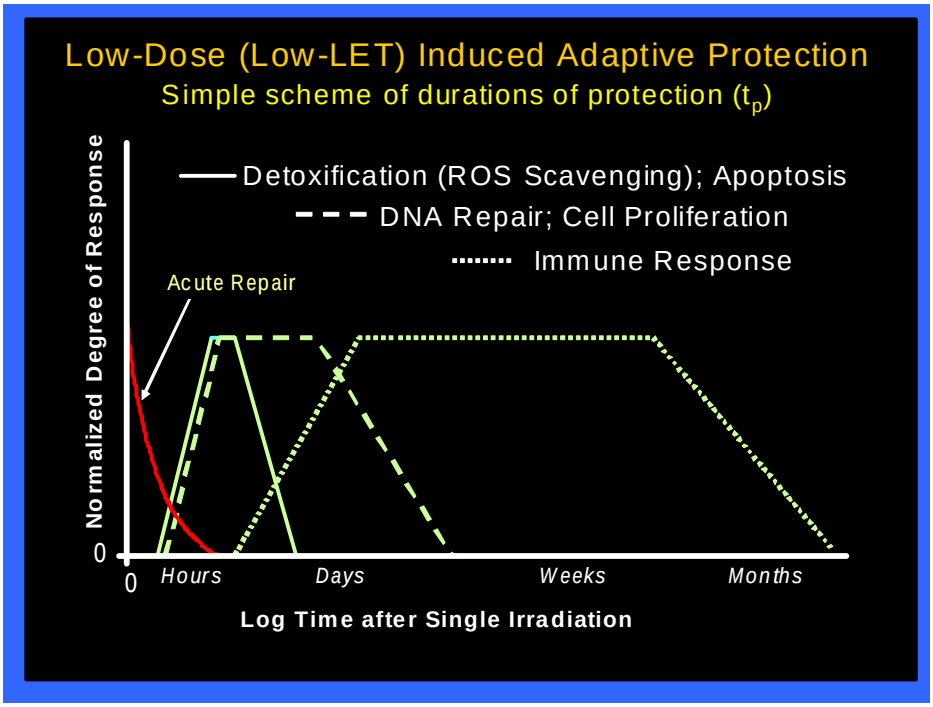
Low-Dose (Low-LET) Induced Adaptive Protection
Simple scheme of durations of protection (t_p)
Detoxification (ROS Scavenging); Apoptosis
DNA Repair; Cell Proliferation
Immune Response
Acute Repair
Normalized Degree of Response
0
0
Hours
Days
Weeks
Months
Log Time after Single Irradiation

Fig. 3

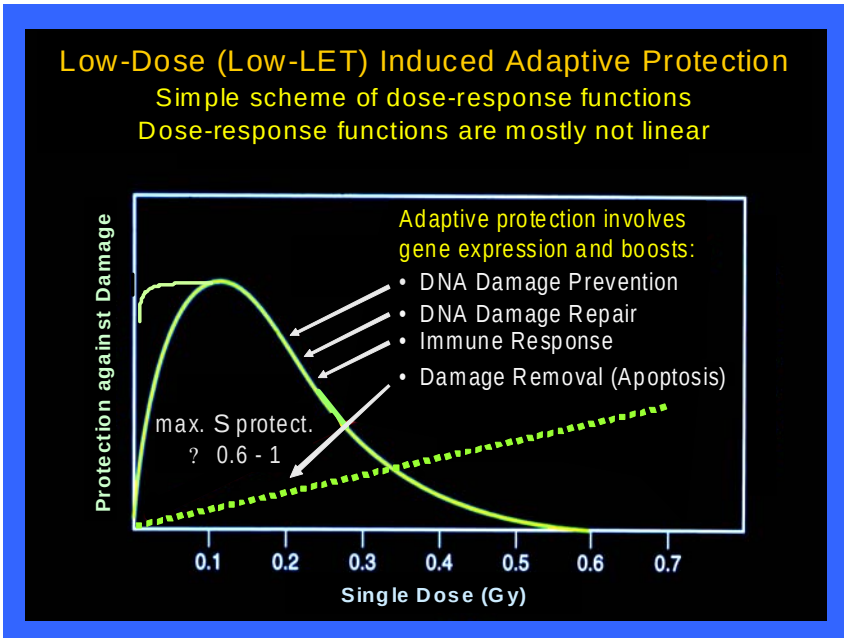
Low-Dose (Low-LET) Induced Adaptive Protection
Simple scheme of dose-response functions
Dose-response functions are mostly not linear
Adaptive protection involves
gene expression and boosts:
• DNA Damage Prevention
• DNA Damage Repair
• Immune Response
• Damage Removal (Apoptosis)
max. S protect.
? 0.6 - 1
Protection against Damage
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Single Dose (Gy)

Fig. 4

reactions or from normal background radiation can trigger reactions commonly addressed as cellular oxidative stress responses. These involve cell signaling of many kinds some with consequences of cellular damage or benefit. They include defense mechanisms, repair of DNA and cell structures, changes of cell cycle times, induction of apoptosis and immunogenic alterations leading, for instance, to immune stimulation (Finkel and Holbrook, 2000; Feinendegen and Neumann, 2000). To what degree low-dose initiated damage or benefit from non-destructive homeostatic perturbations with subsequent adaptive protection prevails depends on species, tissues and cells (Finkel and Holbrook, 2000; Feinendegen and Neumann, 2000; Feinendegen, 2002), determined by the unique genetic make-up of the individual. In this context, normal background irradiation should be seen also participating in keeping tissue homeostasis (Feinendegen, 2002).

Adaptive protection at low-level radiation exposure in risk assessment

The various mechanisms of adaptive protection need to be considered when it comes to assessing risk especially of cancer induction from low-level exposure. To do this coherently and effectively, one should try to adopt a model into which all the phenomena that modulate low-dose responses can be accommodated. Various types of models offer inputs regarding probabilities of cell transformation, clonal expansion, cell removal by different routes; and, thus, serve as insights into mechanisms of radiation induced oncogenesis. Such models are the two-stage clonal expansion model and its modifications (Moolgavkar and Knudson 1981; Schöllnberger *et al.*, 2005). However, most health-physics models pay insufficient attention to the broad range of biological response phenomena predominant in the process of damage propagation from the basic molecular level to increasingly complex and hierarchical levels of biological organization. Other types of model approaches embrace a broader range of biological protection by low doses and appear to come closer to this aspect of biological reality **(**Feinendegen *et al.*, 1995; Feinendegen *et al.*, 1999; Feinendegen *et al.*, 2000; Feinendegen *et al.*, 2004; Heidenreich and Hoogenweem, 2001; Scott 2004; Leonard 2007).

One current model presentation adheres to an approach that was initially introduced by the first author and his collaborators briefly and schematically in 1995 (Feinendegen *et al.*, 1995) It has become more sophisticated over the years but retains its broad appeal to include all aspects and newer findings by fully incorporating the dual effect of low doses and dose rates in both causing damage and in providing for and up-regulating damage prevention by way of adaptive protection against damage propagation to higher levels of biological organization towards clinical disease (Feinendegen *et al.*, 2007; Feinendegen *et al.*, 1995; Feinendegen *et al.*, 1999; Feinendegen *et al.*, 2000; Feinendegen *et al.*, 2004).

The basis of this more recent approach is schematically illustrated in **Figure. 5**. There appear to be two types of risks that are essential in the assessment of a lethal outcome of radiation- induced cancer, or any other late radiogenic disease. First, there is the risk of introducing dangerous damage at the DNA level. This first step largely depends

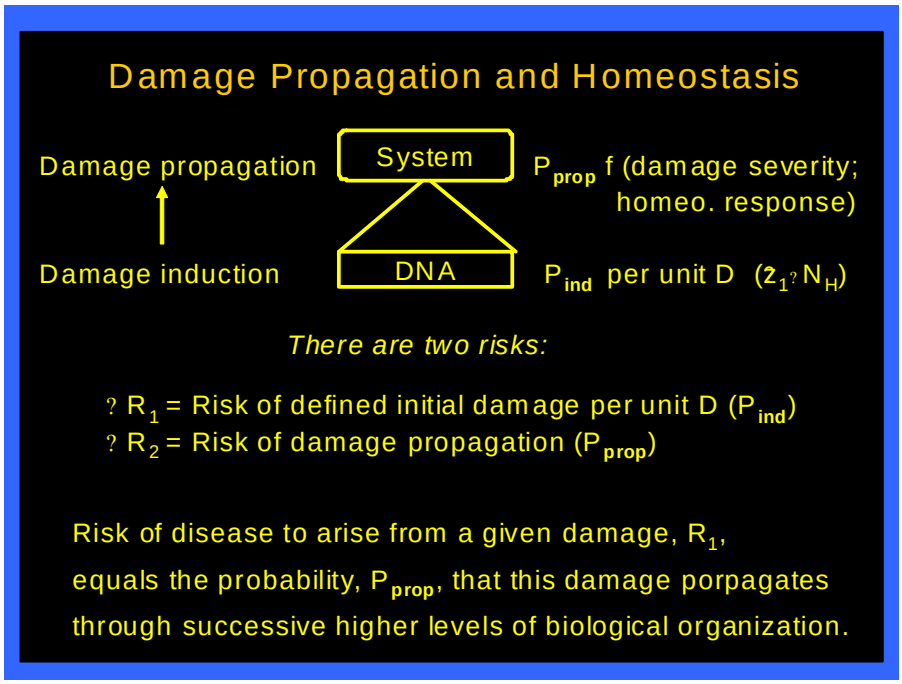
Damage Propagation and Homeostasis
Damage propagation
System
P_{prop} f (damage severity; homeo. response)
Damage induction
DNA
P_{ind} per unit D (λ_1? N_H)
There are two risks:
? R_1 = Risk of defined initial damage per unit D (P_{ind})
? R_2 = Risk of damage propagation (P_{prop})
Risk of disease to arise from a given damage, R_1, equals the probability, P_{prop}, that this damage porpagates through successive higher levels of biological organization.

Fig. 5

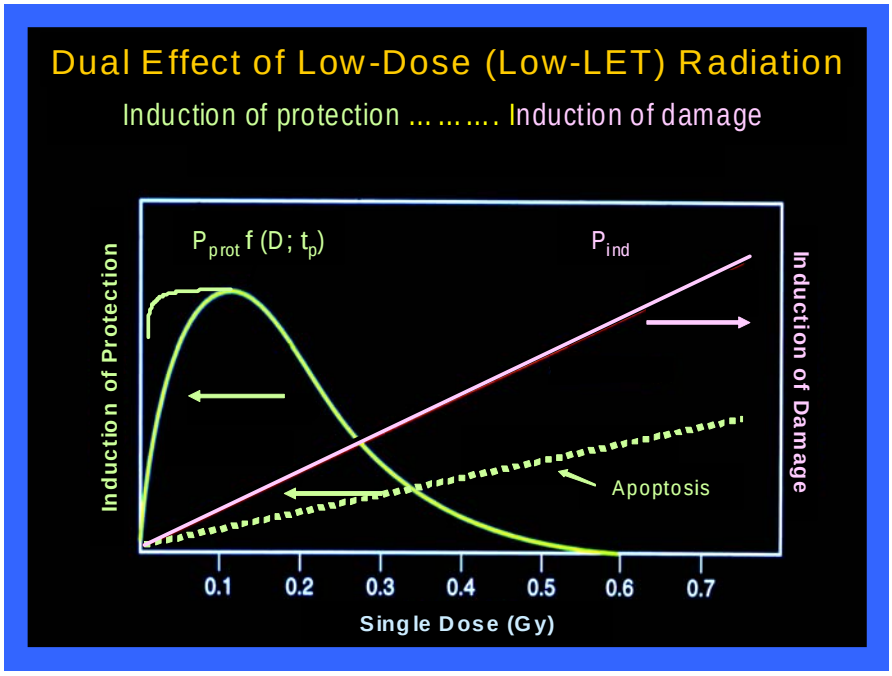
Dual Effect of Low-Dose (Low-LET) Radiation
Induction of protection …… Induction of damage
P_{prot} f (D; t_p)
P_{ind}
Induction of Protection
Induction of Damage
Apoptosis
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Single Dose (Gy)

Fig. 6

on stochastic generation, and primary and secondary interactions of energy depositions at sensitive cell sites, such as the DNA-histone complex. The probability of incidence of a radiogenic damage in the DNA of a given cell rises proportionally to dose over a certain dose range. If one or more of particular types of DNA damages would lead one or more cells to acquiring the potential of causing severe consequences for their host organ/tissue and whole organism, then the question of protective reactions eventually at any organizational level arises. If no protective mechanisms existed or if such mechanisms would operate at a constant rate in the organism, the degree or extent of the initial damage could by simple probabilities of damage incidences linearly determine the degree or extent of resultant disease. This is, as stated above, indeed the inherent, but unproven, assumption used currently by radiation epidemiologists in deriving cancer risk from data as a function of absorbed dose.

However, the second risk is that the primary damage will propagate to higher levels of biological organization. Both the immediate barriers and their adaptive up-regulations at the various organizational levels in response to non-disruptive homeostatic perturbations often operate in a non-linear manner. They involve defined mechanisms throughout their effective responses. This second risk depends, thus, on complex cellular responses at the various levels of the organism and is largely controlled by responding gene expression and many other parameters such as the immune status.

In this context, it is crucial to note that once adaptive protection reactions have begun to function at and between the various levels of biological organization, they may operate also against damages of similar types but of different origins, be they radiogenic or non-radiogenic (Feinendegen *et al.*, 1995; Wolff *et al.*, 1988). Therefore, quantities and qualities of various types of DNA and other damage from radiation exposure and of non-radiogenic origin need to be compared and be related to the corresponding incidence of cancer induction (Pollycove, and Feinendegen, 2003). If one considers the induction of DNA double-strand-breaks as a relevant initial step towards carcinogenesis, which is not proven yet, it is worth to compare the different sources of such double-strand-breaks in human cells. An attempt to quantify DNA damage from normal background radiation and of non-radiogenic, mainly metabolic origin has come to predict that at the level of single cells there are per day on average about a thousand times more metabolically caused DNA double-strand-breaks than double-strand-breaks caused by background radiation (Pollycove, and Feinendegen, 2003). A similar relatively high ratio of double-strand-breaks from the two sources was confirmed subsequently by experimental observations from various laboratories (Rothkamm and Löbrich, 2003; Sedelnikova *et al*., 2004). Considering the relatively large number of more severe types of radiogenic DNA double-strand-breaks compared to that caused by normal metabolism (Ward, 1988; Hada and Sutherland, 2006), it is not surprising in this working hypothesis and irrespective of defined oncogenic mechanisms that human cancer induction from metabolic sources is not about a thousand times but only about 30 to 50 times more frequent than the calculated radiogenic cancer at normal radiation background levels, provided the

assumed linear dose-risk relationship applies to the calculation of both incidences. The undisputed relatively large incidence of non-radiogenic metabolic or "spontaneous" cancer lets it appear justified to assume that the low-dose induced up-regulations of protection operate mainly against the early steps of spontaneous cancer risk (Feinendegen *et al*., 1995; Feinendegen *et al*., 2005; Pollycove, and Feinendegen, 2003; Feinendegen, 2003). Here again, experimental evidence agrees with this assumption (Tubiana *et al*., 2006; Azzam *et al*., 1996; Redpath and Antoniono, 1998; Mitchel *et al*., 2003; Tapio and Jacob 2007).

The simple model used in this presentation to demonstrate the effect of protection induction includes the two risks mentioned above, that of R_1 relating to damage induction, with the risk coefficient, P_{ind}, and that of R_2 relating to damage propagation ascending through the consecutive higher levels of biological organization towards causing finally clinical disease

Risk coefficient P_{ind} is taken to be the probability of radiation induced serious DNA damage per unit dose D of radiation, which hypothetically would evolve to clinical cancer assuming no or some constant rate of protection irrespective of the value of D. This probability is assumed to be constant per unit D over a certain dose range. Coefficient P_{ind} thus conforms in this simple model to the conventional proportionality constant α in the well known expression of the linear dose-risk function: $R = \alpha D$.

The coefficient of the second risk R_2 is here expressed by the term $P_{prot} \bullet f (D, t_p)$. It gives the fractional cumulative probability of the protection up-regulation per unit D inhibiting damage propagation, as a function of D and of the duration of protection effectiveness t_p: as illustrated in **Figures 3 and 4.** An $f (D, t_p)$ value here of 0 means no adaptive protection, and a value of 1 means full protection of damage propagation by adaptive responses in the system. Hence, R_2 describes the diminution of damage propagation.

As outlined above, the low-dose induced protections at the various levels are seen to operate against propagation of damager, especially that of DNA, of any origin, be it non-radiogenic or radiogenic, that threatens to develop into clinical cancer. Introducing the variable P_{spo} to describe the prevalence of spontaneous, *i.e.*, non-radiogenic, serious cell damage causing cancer per individual at the time of observation, and adding the term $P_{ind} D$ to indicate that $P_{prot} \bullet f (D, t_p)$, of course, also affects $P_{ind} D$, the value of $P_{prot} \bullet f (D, t_p)$ $(P_{spo} + P_{ind} D)$ describes the total, beyond the immediate protection remaining probabilities of dose dependent inductions of protection against propagation of any cell and DNA damage to cause cancer. This emphasis on the dual effect of low-dose radiation is consistent with experimental observations on mechanisms leading to the individual responses, **see Figure 6.**

The following mathematical expression simplifies the approach published elsewhere in microdosimetry terms (Feinendegen *et al.*, 1995; Feinendegen *et al.*, 2000; Feinendegen *et al.*, 2004; Feinendegen, 2003). The net risk of cancer, R, at a given level of D is then in this model the sum of

the two separate risks R_1 and R_2, as defined above:

$$R = P_{ind}\, D - P_{prot} \bullet f(D, t_p)\,(P_{spo} + P_{ind}\, D)$$

Note that with increasing D, the term $P_{prot} \bullet f(D, t_p)\,(P_{spo} + P_{ind}\, D)$ tends towards zero notwithstanding the protective contribution by apoptosis **(see Figure 4**). Moreover, the term also reaches zero with t_p becoming too short for $P_{prot} \bullet f(D, t_p)$ to develop or operate **(see Figure 3).** Thus, as discussed below, in case of protracted exposure at low dose rates individual energy deposition events occur at certain time intervals per exposed micro-mass depending on dose rate per radiation quality. In such situations, the time t_p may be too short not only for $P_{prot} \bullet f(D, t_p)$ to develop and act, but also $P_{ind}\, D$ may be augmented through interference with acute repair of the primary damage (Feinendegen and Graessle, 2002).

The graphic display of the model in **Figure 7** in principle combines data from experimental observations, referred to above. The straight line expresses R_1 and shows the linear increase of radiation-induced DNA damage causing clinical cancer assuming no or a constant rate of protection irrespective of D. This display negates here, for ease of reading, the possibility of contributions of secondary DNA damage, be it from bystander effects or through genomic instability. This plotting adheres to the linear-no threshold-hypothesis conventionally expressed as $R = \alpha\, D$. - The lower curved line illustrates the inverse expression of the second dose-risk function $R_2 = P_{prot} \bullet f(D, t_p)\,(P_{spo} + P_{ind}\, D)$, showing damage propagation being inhibited by the overall effect of low-dose induced protection against P_{spo} and $P_{ind}\, D$, with P_{spo} being here the predominant term in normal individuals. The difference between these two dose-risk functions, $R_1 - R_2$, yields the net dose-risk function R which the solid middle line illustrates. For individual application, this model needs amendment with individually measured data, which are expected to vary between species, individuals, and cell types because of individual genetic control. The result of this model approach, nevertheless, conforms to a large set of experimental and epidemiological data, and is in line with the concept of hormesis (Tubiana *et al*., 2006; Azzam *et al*., 1996; Redpath and Antoniono, 1998; Mitchel *et al*., 2003; Tapio and Jacob 2007; Pollycove and Feinendegen 2001).

Low dose rate exposure

The present short treatise on the consequences of the dual effects of low-level exposure to ionizing radiation at the basic molecular level, and on damage propagation in complex biological organisms would be incomplete without reference to low dose rate exposure. As alluded to above, dose rates may be described in terms of mean time intervals t_x between consecutive energy deposition events per exposed micro-mass, *i.e.*, between consecutive micro-dose events, for a given radiation quality (Feinendegen and Graessle, 2002; Feinendegen *et al.*, 1985; Feinendegen *et al.*, 1994). An example is given here from studies (Yamamoto *et al*., 1998) in which mice were chronically exposed to tritiated water throughout life. As shown in **Figure 8,** thymic lymphoma induction and life shortening only appeared at dose rates above 1 mGy per day. This dose rate at chronic tritium exposure corresponds to about 1 micro-dose event of 1mGy occurring per micro-mass within less

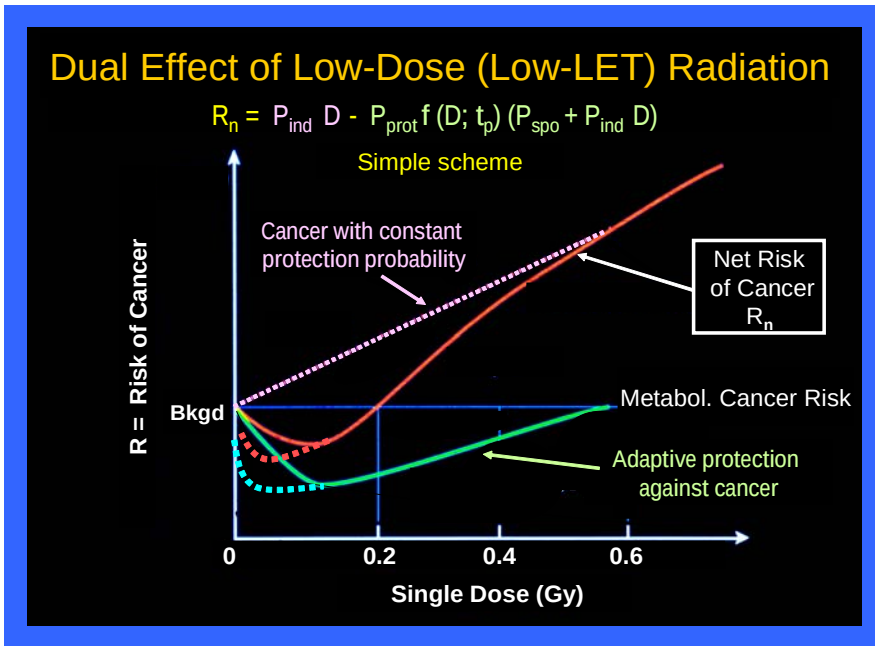
Dual Effect of Low-Dose (Low-LET) Radiation
$R_n = P_{ind} D - P_{prot} f(D; t_p) (P_{spo} + P_{ind} D)$
Simple scheme
Cancer with constant protection probability
Net Risk of Cancer R_n
R = Risk of Cancer
Bkgd
Metabol. Cancer Risk
Adaptive protection against cancer
0
0.2
0.4
0.6
Single Dose (Gy)

Fig. 7

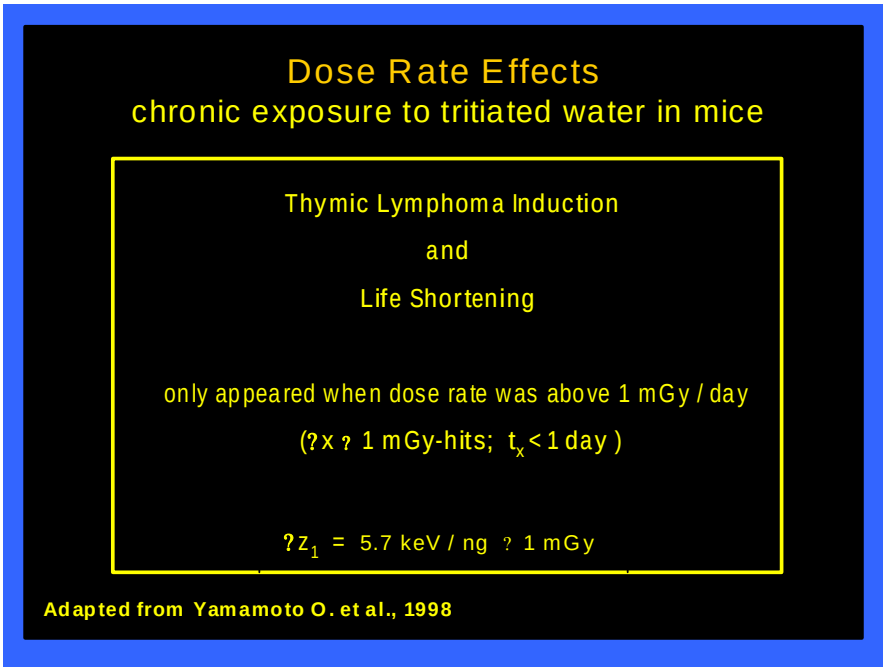
Dose Rate Effects
chronic exposure to tritiated water in mice
Thymic Lymphoma Induction
and
Life Shortening
only appeared when dose rate was above 1 mGy / day
(?x ? 1 mGy-hits; t_x < 1 day)
$?z_1$ = 5.7 keV / ng ? 1 mGy
Adapted from Yamamoto O. et al., 1998

Fig. 8

than 1 day, *i.e.*, at a t_x shorter than 1 day (Feinendegen *et al.*, 2007) There was no thymic lymphoma and no life shortening when t_x was longer than 1 day. Such assessment complements other reports on the effects of chronic low dose rate exposures to a given radiation quality (Pollycove and Feinendegen, (2003; Scott, 2004; Feinendegen and Graessle, 2002).

Occupational exposures in humans, for instance, usually deliver much lower dose rates as discussed above, and thus provide for relatively long t_x. Other examples are in many epidemiological data on accidental or medically directed chronic irradiations of humans. At an optimally long t_x immediate and adaptive protections are expected to fully operate within the cell's capacities (Feinendegen and Graessle, 2002). This is likely the reason for the repeated epidemiology observation of a reduced cancer incidence below the background incidence at chronic low dose rate exposures (Tubiana *et al.*, 2006; Pollycove and Feinendegen, 2001; Luckey, 1980). One recent reference relates to the analysis of the mortality of 45 468 Canadian nuclear power industry workers after chronic low-dose exposure to ionizing radiation (Zablotska *et al.*, 2004). This paper quotes: "For all solid cancers combined, the categorial analysis shows a significant reduction in risk in the 1-49 mSv category compared to the lowest category (<1 mSv) with a relative risk of 0.699 (95% CI: 0.548, 0.892). Above 100 mSv the risk appeared to increase".

Moreover, the recently published analysis of cancer incidence in 407,391 nuclear workers in 15 countries showed no elevated excess relative risk with relatively narrow confidence intervals below cumulative doses of about 150 mSv. In fact, the data also do not contradict a reduction of relative risk at these low doses (Cardis *et al.*, 2007).

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