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Significance of basic and clinical research in radiation medicine: challenges for the future

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Abstract. Tissue effects are the consequences of cellular reactions and responses. This review deals with cellular responses to low absorbed doses of ionising radiation, which are not readily predictable by extrapolation of responses observed at high doses. One of the reasons for this unpredictability is the relationship between the relatively low density of particle tracks in tissue at low radiation doses with their generation of largely stochastic ionisations and excitation of constituent molecules, and moreover, bursts of reactive oxygen species (ROS). The other reason is the abundant and constant generation of ROS and other endogenous toxins, on top of which low dose radiation acts. At low doses, a dual effect on cellular DNA occurs: one concerns damage, whilst the other brings adaptive protection which develops within hours and may last for days to months. Radiation-induced apoptosis and immune responses add to protection by reducing genomic instability and eliminating pre-damaged cells from tissues. Protective responses express adaptive responses to suprabasal metabolic perturbations and also mimic oxygen stress responses. Analysis of the consequences of this low dose scenario, which also applies to background irradiation, predicts that the linear no-threshold hypothesis is scientifically unfounded. In cases of protracted or chronic irradiation, the time interval between repetitive particle tracks in a defined biological target at a given dose rate may determine to what degree damage or adaptive protection prevails. Tissue failure following chronic exposure at low dose rate appears to result from the rather sudden shift from a low to a high ratio of radiogenic damage to protection against endogenous damage, within the net response of largely still elusive signalling networks in cells and tissues.

Introduction

The theme of this Workshop has a strong clinical connotation yet embeds many questions pertinent to the pathogenesis of radiogenic multi-organ insufficiency and eventual failure.

This may not sound very new and challenging in view of the enormous wealth of data on radiation-induced acute diseases, as they have been repeatedly summarised following the seminal book "*Mammalian Radiation Lethality: a disturbance in cellular kinetics*" in 1966 [1], which is a landmark publication still today. Whereas for many years acute and life-threatening illnesses following irradiation were almost exclusively linked to high dose exposure, such as from the atomic bombs in Japan, acute serious illness may also arise from chronic exposure to relatively low doses of low-LET (linear energy transfer) and high-LET radiation.

Bone marrow failure developed in various animal species during chronic low dose whole body γ-irradiation when the accumulated dose reached a certain level [2], which depended on the dose rate [3, 4]. For instance, in dogs exposed at 37.5 mSv per day, survival times varied between 1 year and 8.5 years, with a mean reduction in life span by 8% compared with control dogs. Tissue failure occurred rather abruptly within a few days, indicative of a tolerance threshold when excess cell loss from the stem cell pool reached a critical value [5]. At dose rates below 37.5 mSv per day, survival times varied widely, similarly to control dogs, with a mean survival time close to 10 years. Death was associated with haematopoietic failure, neoplasia (solid tumours, leukaemia) and other causes. These animals retained full capacity for erythropoiesis, myelopoiesis and megakaryocytopoiesis almost throughout life. For example, dogs exposed to a dose rate of 10 mSv per day and having a survival time of 3000 days with death from radiation-induced tissue failure, accumulate a total dose of 30 Sv at time of death. With a D_0 value of less than 1 Gy for haematopoietic progenitor cells, the dose reduction factor would be around 30 and would not be explainable simply by repair of sublethal cell damage with concomitantly altered cytogenetics and modified cell cycle characteristics in the exposed dogs [2]. Indeed, an adaptive response in haematopoietic progenitor cells with changes in radiation sensitivity appeared in that the D_0 value of these cells tested *in vitro* increased by a factor of more than two [3].

The experimental observations in animals appear to be similar to clinical data from humans who have been accidentally exposed to chronic to γ-radiation for more than 150 days at dose rates ranging up to 10 mSv per day [4]. The obvious capacity of cell renewal systems in mammals to adapt to chronic irradiation at very low dose rates poses most interesting questions in basic cell research and moreover, has immediate relevance to the demands for radiation protection.

This paper aims to briefly summarise the expected demands for basic rather than clinical research in order to understand the biological and health effects of chronic exposure to low doses of ionising radiation. In doing so, three aspects deserve special attention, as illustrated in Figure 1: (a) radiation as a toxin to cell metabolism; (b) cellular responses to low doses and dose rates; and

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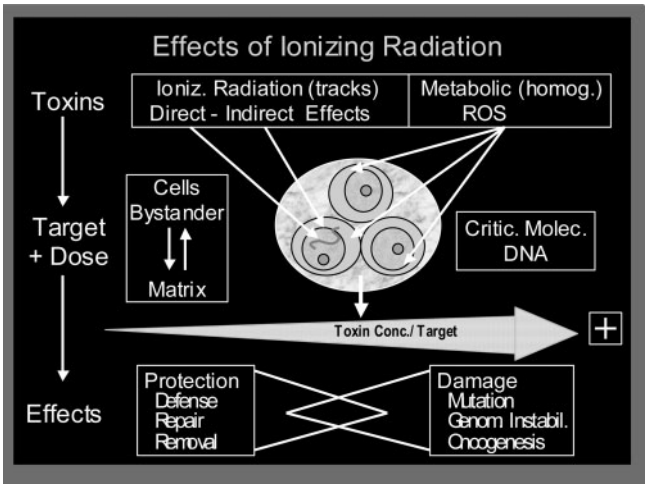


Figure 1. Ionising radiation is a toxin to specific targets. Cells trigger tissue effects that vary with types of target response. Effects in cells and tissues depend on the degree of system perturbation. Low perturbations initiate adaptive responses; high perturbations bring damage or death. Note that radiation effects are partly comparable with effects of reactive oxygen species (ROS).

(c) the relationship between tissue effects from tissue-constituent cells and absorbed dose.

Radiation as a toxin to cell metabolism

Ionising radiation and endogenous reactive oxygen species

Absorption of ionising radiation of any type or quality occurs by way of particle tracks. In the case of penetrating radiation, these tracks arise stochastically throughout the exposed tissue [6]. In the case of exposure to internal emitters, the distribution of particle tracks is determined by the distribution of the emitter in the tissue [7]. The higher the radiation fluence or number of particle emitters in a given tissue mass, the more concentrated are the particles in the exposed site.

The energy deposited per track in biological tissue creates a multitude of ionisations and excitations, and water molecules are always involved. These cause at least two types of molecular alterations: (a) by unpredictable or stochastic direct effects on many different molecular constituents of cells and the extracellular matrix; and (b) by a number of molecular species following the radiation-induced hydrolysis of water, summarily called reactive oxygen species (ROS). These ROS are similar to those radical compounds that result from normal oxygen metabolism [8]. Thus, each particle track in a biological tissue may on the one hand be compared with a physical tool to locally inject a burst of a certain amount of ROS into the system in addition to the metabolically produced ROS, and on the other hand may be considered as producing at times clustered structural alterations in many different molecular constituents of the tissue by direct effects along its track. These altered compounds are provocative to biological systems, although this is different from the primary effects of ROS. Indeed, ionising radiation should be viewed as creating a dual set of potentially toxic compounds in a manner that hardly ever applies to conventional chemical toxins irrespective of whether they are produced endogenously by metabolic reactions or whether they enter biological systems from external sources. An important distinction between radiation-induced potential toxins and those toxins commonly encountered in toxicology is their distribution in biological systems. Even at low amounts, conventional toxins administered to a system are usually more homogeneously distributed than particle tracks from low doses of ionising radiation. Mechanisms of their biological effects vary accordingly throughout tissue. However, common to both is the principal distinction between triggering event sites and sites where biological effects become manifest, and these two sites may be quite far apart in terms of microscopic dimensions.

Rate of generation of endogenous and radiogenic ROS

It is generally accepted that both endogenously generated ROS and ROS from energy deposition from ionising radiation are similar although not necessarily identical. They both have a short life span, and they may be perpetuated through secondary reactions, for instance with polyunsaturated fatty acids that again may cause tertiary biochemical reactions [9]. The particular difference between metabolic and radiogenic ROS lies in the frequency of their occurrence and their compartmentalisation. In a normal mammalian cell, ROS are produced constantly and abundantly by different reactions, with mini bursts occurring probably at rather frequent and changing time intervals, partly in response to certain ligand–receptor interactions [10, 11] (see Figure 2). A network of antioxidant enzymes maintains a homeostatic steady-state concentration of ROS in cells within a physiological range. Approximately 10^9 ROS may escape the mitochondria into the cytoplasm per average cell per day [12]. This would generate approximately 11 600 ROS per second per average cell via mitochondrial escape alone, with this level changing depending on the extent of oxidative metabolism. Other mechanisms of physiological ROS generation from oxidative metabolism add to this production rate at different sites [13].

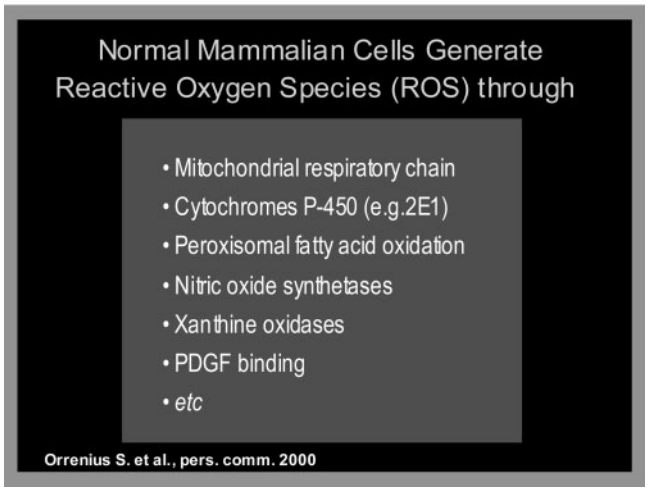


Figure 2. Generation of reactive oxygen species by normal mammalian cells. (person. comm. S Orrenius, 2000).

Radiation-induced bursts of ROS are likely to be larger than the metabolically produced mini bursts. The former tend to be suprabasal ROS bursts and occur with a rate that is dependent on the dose rate, and their size distribution depends on the radiation quality. As explained in more detail below, low-level natural background radiation randomly generates in the exposed tissue on average several particle tracks, *i.e.* energy deposition events, per nanogram per year. For instance, an assumed background dose rate of 2 mGy of 100 kV X-rays causes two events, and thus ROS bursts, per nanogram mass in the body on average approximately every 6 months. Such repetitive events may affect the hit tissue mass, be they cells or the intercellular matrix, as well as the non-hit neighbouring cells through intercellular and matrix signalling [14, 15].

Radiogenic ROS bursts

Regarding average ROS burst sizes, approximately 30 eV are used per creation of a single ROS by radiolysis of water, and 1 mGy per nanogram expresses the absorption of 6.24 keV in this mass [6]. Approximately 70% of the tissue-absorbed energy from low-LET radiation is dispensed in the radiolysis of water. Microdosimetry analyses show that the individual energy deposition event per nanogram of tissue from ^{60}Co γ-radiation absorption delivers on average a dose of 0.3 mGy to this mass. This dose amounts to the generation of approximately 45 primary ROS along the average electron track within less than a microsecond. For ^{137}Cs γ-irradiation, the equivalent dose is 0.4 mGy, and a burst of approximately 60 primary ROS would result per average energy deposition event. The comparative values for 250 kVp X-rays are an average dose of 0.9 mGy and a burst of 130 primary ROS; and approximately 150 ROS per burst per nanogram result from an average dose of 1 mGy of 100 kVp X-rays. The average number of primary ROS per burst from absorption of densely ionising α-particles per nanogram reaches much higher values, ranging up to some tens of thousands per α-particle, again within less than a microsecond, in addition to the relatively high incidence of direct molecular interactions. However, the frequency of such high-energy deposition events per nanogram of tissue is extremely low at low dose irradiation [16].

The parallel creation of two types of molecular alterations in irradiated biological systems has been known since the early days of radiation biology, in particular with respect to both of them causing DNA damage at high dose irradiation and to radiation therapy. However, at low doses and low dose rates, where radiation-induced DNA damage has a very low probability, as discussed below, it is necessary to consider radiogenic ROS separately from the constantly and abundantly produced metabolic ROS and their corresponding biological effects.

Cellular responses to low doses and dose rates

With regard to the two categories of primary molecular changes from absorption of ionising radiation in biological tissue, namely damaged molecules and burst generation of ROS, it seems justified to discuss cell responses to both categories separately with reference to metabolically, *i.e.* endogenously, generated ROS.

Radiogenic cell damage

Ionising radiation affects cellular constituents at random both directly and by way of ROS. The DNA is generally accepted to be the critical molecule even at low doses, and accumulated DNA damage may have serious consequences, such as life shortening and increased incidence of cancer [2]. Increase in DNA damage is proportional to absorbed dose. If one extrapolates measured DNA and chromosome damage from high to low doses, then 1 mGy of low-LET radiation, such as 100 kVp X-rays, generates, as discussed further below, on average per 1 nanogram, *i.e.* per average cell mass: 1 energy deposition event; approximately 150 ROS; 2 DNA alterations of any kind; 10^{-2} DNA double-strand breaks (DSBs); 10^{-4} chromosomal aberrations; and the probability of an oncogenic transformation with lethal outcome of approximately 10^{-13} to 10^{-14} [15, 17, 18] (see Figure 3). In other words, a sizeable ROS burst per cell is accompanied by a low probability of serious DNA damage. Moreover, the ratio of the incidence probabilities for radiation-induced lethal cancer and the corresponding DSBs is approximately 10^{-11} to 10^{-12}. This means that the statement that even one DSB causes a lethal cancer to develop from the affected cell is unreal, scientifically unfounded and indeed irresponsible. Nevertheless, it is a challenge to understand the reason for the extremely low probability of DSBs to cause lethal cancer. The cited values are calculated averages from irradiated multicellular systems. This implies that any bystander phenomenon that may have occurred is co-registered and expressed in the observed values from which these calculations were made [19].

Effects of endogenous ROS

With regard to metabolically produced ROS, significant biological functions and effects in terms of signalling and damage depend on ROS concentration and the rate of ROS production at a given biological site [20–23]. With

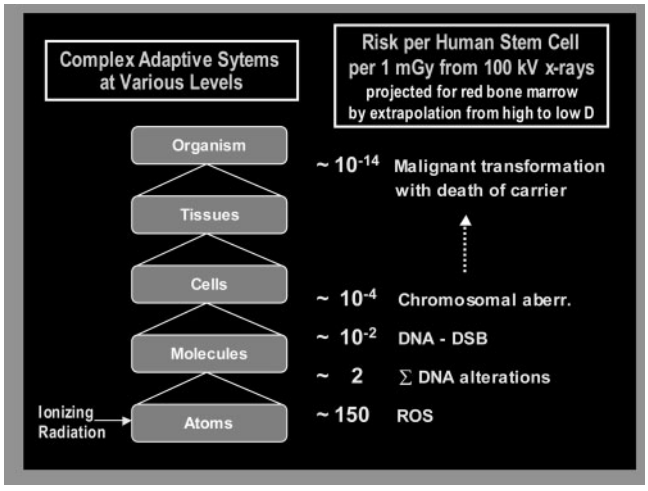


Figure 3. Risk per human stem cell per 1 mGy from 100 kV X-rays. *D*, absorbed dose; ROS, reactive oxygen species; DSB, double-strand breaks.

regard to damage, metabolically generated ROS are potential toxins depending on their local concentration and compartmentalisation, for instance causing oxidation in the DNA (oxiadducts) with various types of strand breaks, as well as oxidations of other molecules especially in proteins, with many being potentially carcinogenic and accelerating ageing [9, 11, 12, 24–26]. The ROS involved in altering DNA in HeLa tissue culture cells appeared to come less from the mitochondrial respiratory chain than from other biochemical reactions in the cell, and the rate of DNA base changes occurring per day from ROS amounted to approximately 10^5 to 10^6 [13]. This value agrees with calculations on the basis of a careful search of the literature on ROS-generated DNA changes in many different cell systems. The result showed approximately 10^6 DNA oxiadducts to arise per day per nuclear genome in mammalian cells [12].

Ratio of DNA damage from endogenous ROS and radiation

The relationship between DNA damage from low dose irradiation and from endogenous metabolic sources, mainly ROS, has been the topic of extensive discussions [12]. As illustrated in Figure 4, the estimated ratio of total induced DNA alterations, including base changes, single-strand breaks (SSBs) and DSBs, from endogenous sources, mainly ROS, to those from background γ-irradiation of 1 mGy per year is approximately 10^7, and the corresponding ratio for DSBs is approximately 10^3. It also emerges that at low-LET irradiation the probability of radiation-induced DSBs per primary DNA alteration of any type is 10^5 times higher than that caused endogenously. These data attest on the one hand to the fact that endogenous DNA damage far outweighs radiation-induced DNA damage at background level exposure, and on the other hand that radiation-induced random energy deposition events are far more effective in causing cellular DNA damage than endogenous ROS, probably owing to the topography and spacing of ionisations and ROS along the particle tracks.

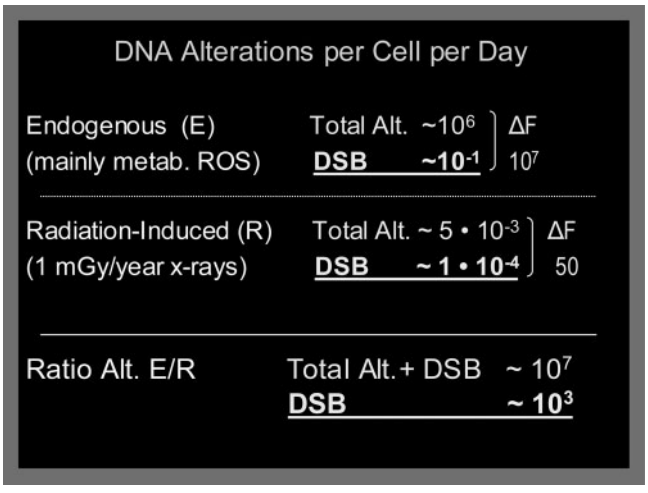


Figure 4. DNA alterations per cell per day from low dose irradiation and from endogenous metabolic sources. ROS, reactive oxygen species; DSB, double-strand breaks.

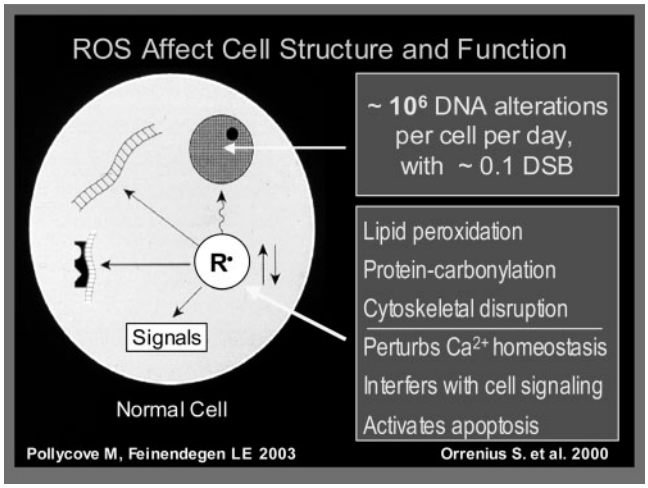


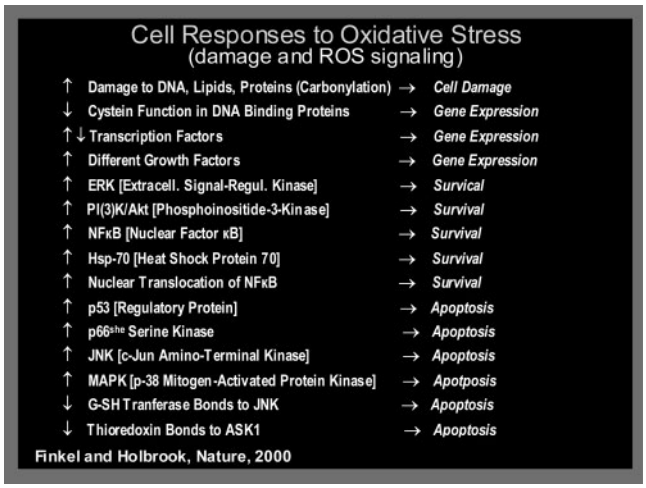
Figure 5. Effects of reactive oxygen species (ROS) in mammals on cell structure and function. DSB, double-strand breaks. (person. comm. S Orrenius, 2000).

Effects of ROS signalling

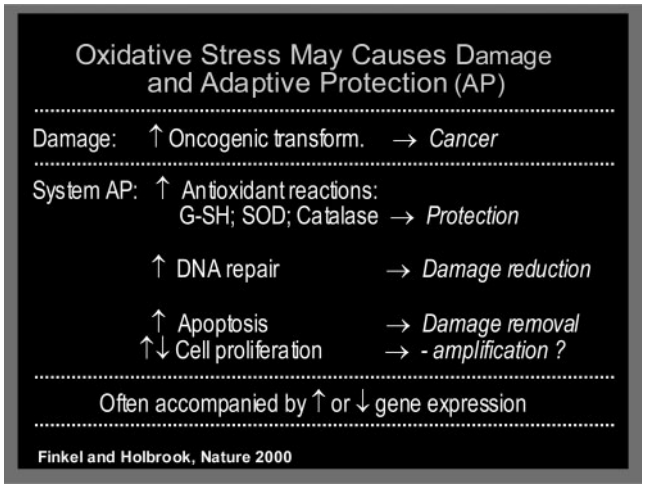
ROS-induced molecular changes, including DNA alterations, may also act through feedback controls and thus add to biological signalling cascades [27, 28]. A particular signalling cascade occurs as a result of oxidative stress. This cascade essentially involves ROS directly by affecting a broad range of reactions including those that regulate gene expression and apoptosis [20–24, 29]. Figure 5 schematically summarises the effects of ROS in mammalian cells. Figures 6a lists a number of metabolic reactions in response to oxidative stress and Figure 6b shows their summary. These reactions include those that defend against an elevated level of ROS in order to facilitate DNA repair and to remove DNA damage, for instance by apoptosis. Whether damage or signalling for protection prevails apparently derives from the rate and size of change of toxin concentration in the target system. Thus, for ROS to induce apoptosis depends on the ROS concentration in the cell (see Figure 7).

Adaptive responses

A sudden moderate rise of toxin concentration in a target tends to elicit stress responses and to stimulate adaptation, usually in terms of protective mechanisms in the sense of hormesis [30]. Adaptive protection, such as shown in Figures 6a and 6b for oxidative stress, constitutes adaptations to a renewed stress situation in biological systems. Adaptive protection also occurs after low dose exposure and during low dose rate exposure to ionising radiation [15, 18, 31, 32]. It is not clear to what degree ROS are involved in the various reactions. An important question arises as to the biological effects of stochastically produced ROS bursts along particle tracks from low dose or background irradiation. These bursts need to be viewed in the context of the physiological role of endogenous ROS production rate and the associated physiological mini bursts. The quantitative relationships between the endogenous steady-state ROS levels with relatively frequent mini bursts and the rarely caused radiation-induced ROS bursts are likely to be different in various cell types and



(a)



(b)

Figure 6. (a) Cell responses to oxidative stress. (b) Summary of responses. ROS, reactive oxygen species.

species. The endogenous ROS appear in cells at specific sites or compartments and are less stochastically spaced than radiogenic ROS [8].

Acute low dose irradiation and adaptive protection

As stated above, biological effects of ROS appear to depend on their rate of generation on top of a steady level, leading to protective signalling and/or damage. Even multiple ROS bursts from low-LET particle tracks, as discussed above, are effective in generating adaptive protection. Here, two principal consequences appear to be relevant for low dose and low dose rate radiobiology. One of these is signalling for adaptive protection to keep damage low and the affected cells alive; this protection operates at low doses and increasingly disappears at doses above approximately 0.2 Gy, *i.e.* at more than about 200 burst of approximately 150 ROS each per nanogram in the case of 100 kVp X-rays, as seen in Figure 8 [33, 34]. This cellular function-saving and life-saving response appears to involve damage prevention by stimulating antioxidant defences, enhanced DNA repair and cell proliferation, especially of immunocompetent lymphocytes. These lymphocytes can then remove cells with appropriate antigen presentation from whatever origin, because they are known also to protect against non-radiogenic cell damage, for instance against cancer cells of various kinds [35–37]. This response also operates to protect against damage accumulation from renewed irradiation at high doses and other radiomimetic toxins [38, 39]. The other consequence is to initiate removal of the affected cells and thus to protect tissue against genomic dysfunction and instability. This second set of adaptive protection appears to demand a level of pre-existing damage in cells, *i.e.* cells being prepared for removal [40]. This applies both to apoptosis and to removal by a stimulated immune system. The prerequisite for inducing apoptosis is to have a certain type and degree of damage to the cell [40, 41]. For the stimulated immune system to operate, the damaged target cells must present the appropriate antigen, which signals cell damage to competent immune cells [35–37, 42]. All these responses appear within hours and may

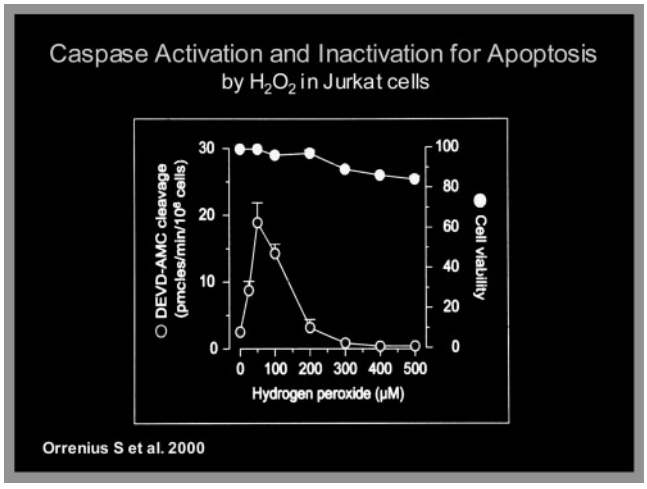


Figure 7. Relationship of level of reactive oxygen species (ROS) and apoptosis in cells: caspase activation and inactivation for apoptosis. (person. comm. S Orrenius, 2000).

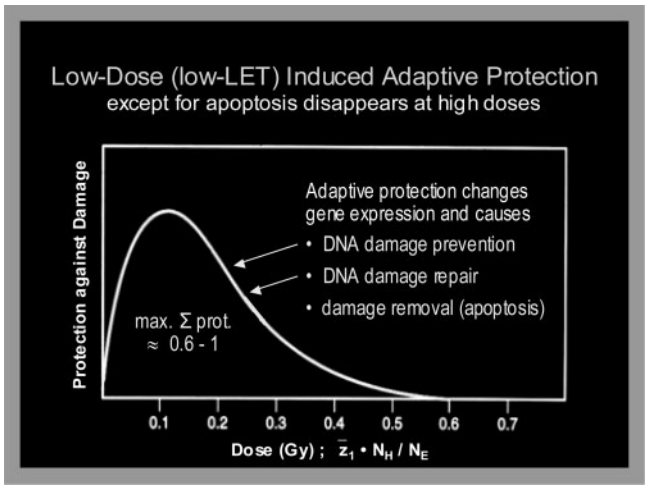


Figure 8. Schematic presentation of dose–effect curves for adaptive protection, except for apoptosis, induced by low dose low-LET (linear energy transfer) radiation exposure.

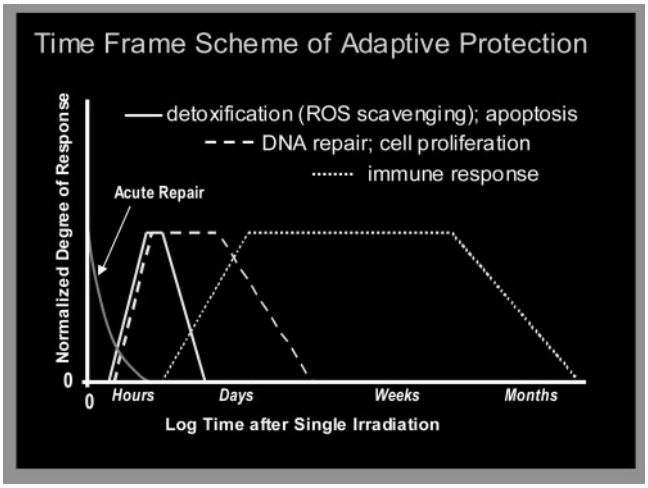


Figure 9. Schematic presentation of duration of adaptive protections. ROS, reactive oxygen species.

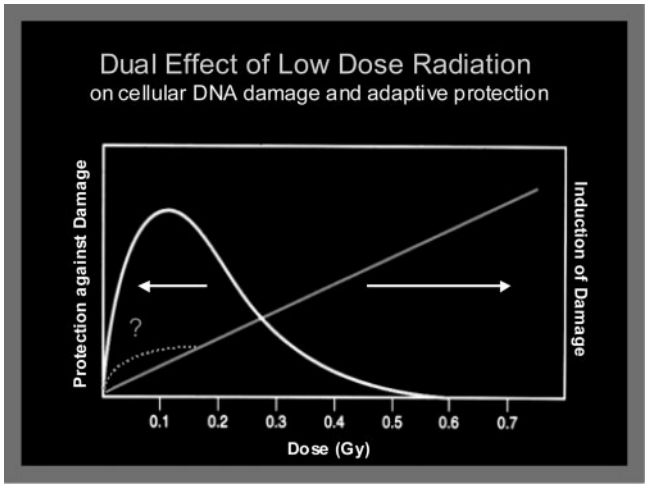


Figure 10. Dual effect of low dose radiation on cellular DNA damage induction and adaptive protection, except for apoptosis.

last up to several weeks or months [15, 18], as shown schematically in Figure 9. As with damage induction, adaptive protection has been observed experimentally in multicellular systems, so that any protecting bystander phenomenon that may have occurred is co-registered and expressed in the observed values.

The pattern of adaptive protection predicts that low dose effects promote primarily damage prevention and damage repair in those cells whose damage has not accumulated to a degree where oxygen stress or other perturbations would preferentially cause apoptosis and immunogenicity. Damage removal by way of apoptosis and the immune response, however, would be the consequence of a protective response that meets cells damaged in some way. So far, little is known about the quantitative relationships between these various protective mechanisms in different cell systems and tissues, yet it is known that they do occur and that they appear to be relevant in understanding the biological effects of low doses and dose rates. Removal of predamaged cells by apoptosis and the immune response may be the most relevant responses to protect a mammalian system, especially against DNA damage-related tissue degeneration and cancer. Therefore, low dose irradiation from background exposure alone could be essential in providing a physiological mechanism for tissue homoeostasis.

The dual effect on cells at low doses

Overwhelming experimental evidence shows a dual pattern of biological responses specifically to low doses of low-LET radiation in many different cell and tissue systems: one response expresses DNA damage, which as discussed above increases linearly with absorbed dose over a certain range; the other response expresses various types of protection against accumulation of DNA damage and keeps the cell alive, and this response category increasingly disappears with doses rising above approximately 0.2 Gy. This dual effect of low dose radiation is shown schematically in Figure 10, in which the potential contribution from possible bystander effects to damage at very low doses is specifically marked.

Chronic low dose irradiation and adaptive protection

The above discussion focuses on responses to a single irradiation. In cases of chronic radiation exposure, repetitive single energy deposition events occur in the micromasses of the exposed tissue. Depending on radiation quality, such repetitive energy deposition events should be seen in the context of repetitive cellular responses to them. By increasing the dose rate, the frequency of energy deposition events per nanogram, and thus per cell average, also increases, and the time interval between two consecutive events per cell shortens. Figure 11 shows the relationship between dose rate and the average time interval between two consecutive events that bring a given dose to the affected nanogram of tissue, here called microdose $\bar{z}_1$, depending on the radiation quality. The figure also lists the dose-rate-dependent frequency of events within 100 ng, *i.e.* cells, per day [43]. The time interval between two events per cell and accordingly, the

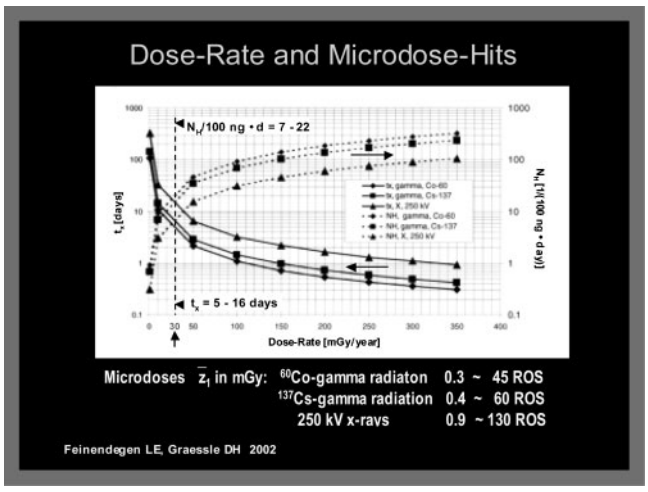


Figure 11. Relationship between dose rate and the average time interval between two consecutive events that bring a given dose to the affected nanogram of tissue, here called microdose $\bar{z}_1$, depending on radiation quality. ROS, reactive oxygen species. The figure also lists the dose-rate-dependent frequency of events within 100 ng, *i.e.* cells, per day. N_H is the number of microdose events in exposed micromasses.

frequency of such events in a given number of cells in a population, may become unfavourable for the optimal development of cell and tissue adaptations to the single event per hit cell and perhaps its neighbours. Under such conditions, two consecutive events per cell within an unfavourable short time frame may cause damage to outweigh any adaptive or protective effect from individual deposition events, and simple or super-additive damage accumulation may occur. Provided damage prevails, the relatively high radiation sensitivity of immature cells will cause their preferential removal from tissue. This removal is likely stochastic and if it affects only a small fraction of the progenitor cells, depending on dose rate, replacement comes from the pool of surviving competent progenitor cells so that a homeostatic equilibrium remains intact with a reduced level of genomic instability. Assuming that radiation sensitivity of progenitor and stem cells does not change during repetitive exposures to energy deposition events at low dose rates within the cells or within their neighbourhood owing to bystander phenomena, this pool would constantly lose a certain fraction of cells and tissue failure would gradually develop. However, failure develops rather suddenly within a few days after relatively high accumulated doses [4]. The abrupt cessation of supply of functioning cells, for instance in the bone marrow, speaks in favour of adaptive mechanisms that increase the radiation resistance in the progenitor pool. This has indeed been observed [3] and appeared also to emerge from studies on adaptive responses in chronically low dose γ-irradiated dogs when the dose rate was 3 mGy per day [44]. The low dose-rate-induced increase in radiation resistance in the pool of progenitor cells is an important issue to study. Knowing more about its cytological origin and mechanisms may also have far-reaching consequences for radiation protection policy. One of the mechanisms involved in stimulating radiation resistance probably mimics the oxygen stress response to repetitive ROS bursts in addition to responses to accumulated DNA damage and other intracellular perturbations from energy deposition events, with cells staying alive, *i.e.* damage kept below the threshold for cell removal, for instance by apoptosis or immune defences.

Challenges

Mechanisms of adaptive protection to single and repetitive irradiation of biological systems are just beginning to be unravelled and much more work needs to be done in the context of general toxicology. To understand biological effects of low doses and dose rates of ionising radiation demands answers to questions that are common in toxicology. These questions also go beyond conventional toxicology because of the complexity of interactions between stochastic sublethal damage to extracellular and cellular constituents with concomitant perturbations, and also because of the more focused actions of ROS loads along particle tracks. In addition, the definition of a low dose rate needs to take into consideration the time constraints of development of adaptive protection for different radiation qualities. These are likely to vary with different cell types and species.

Relationship of tissue effects from tissue-constituent cells to absorbed dose

In attempting to relate tissue effects at low level radiation exposure to the various cellular responses, as described above, the conventional use of tissue-absorbed dose does not satisfy the desire to better understand the genesis of effects per unit dose. This stems in part from the fact that there is a principal difference in the measurement between the absorption of ionising radiation and toxins from endogenous or outside sources. In toxicology, the amount of potential toxin administered to the biological system is usually quantified, for instance in terms of weight or number of molecules of toxin in a biological system, and not in terms of concentration at a given site per unit tissue mass. For radiation, however, absorbed dose D is defined as energy absorbed per unit mass, *i.e.* as concentration at a given tissue site. It is obvious that absorbed dose D in a large exposed tissue mass may be identical to the absorbed dose D in a fraction of this exposed mass, and equal doses then relate to different total energies absorbed in these masses, as illustrated in Figure 12 [45]. Expressing radiation effects as a function of absorbed dose should adjust to the mass that is critical to effect development or where an effect is triggered.

This difference in dosimetry between ionising radiation and general toxins is less problematic at high radiation doses, for instance in radiation therapy. Here the high density of particles in the exposed mass mimics a large number of potentially toxic molecules that are distributed rather uniformly in the exposed volume to be treated or under observation. At low doses, however, the particle tracks are more or less distinct from each other and are often distributed unevenly. They trigger effects at the site of their occurrence, such as in a tissue micromass, *i.e.* cell, and effects may range far beyond the triggering site, for instance in their neighbourhood through secondary toxins and/or signalling molecules depending on the energy deposited at the triggering site [19, 46, 47; pers. comm., BD Michael, 2003]. Even at low levels in a system, toxins are usually more homogeneously distributed in tissue than are particle tracks from ionising radiation, and mechanisms of biological effects vary accordingly throughout the tissue. Common to both, however, is the principal

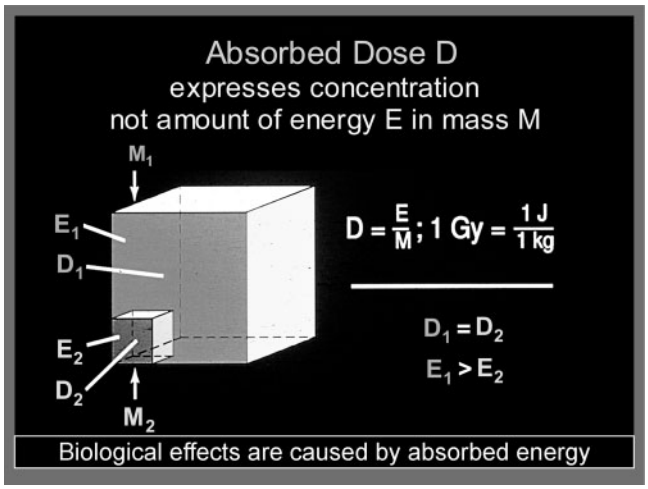


Figure 12. Absorbed dose D expresses concentration not amount of energy E in mass M.

distinction between triggering event sites and sites where biological effects become manifest, and these two sites may be quite distant from each other in terms of microscopic dimensions.

One approach to solving the problem of absorbed dose of ionising radiation at low dose and dose rate exposures has been previously presented and makes use of microdosimetry [15, 19, 48–50]. As presented schematically in Figure 13, tissue-absorbed dose D of a given radiation quality is expressed here as the distribution weighted sum of energies delivered by multiple deposition events in defined micromasses divided by the sum of the exposed micromasses, defined here to be 1 ng each. This mass is an arbitrary choice but corresponds to the average mass of a mammalian cell [16]. The energy deposited by a single particle track traversing a tissue micromass of 1 ng has been denoted by the term ''microdose'', and the event delivering this microdose has been referred to as a ''microdose hit''. Its mean energy is a function of the radiation quality [15, 18]. Large values of absorbed dose of a given radiation quality in the tissue create large numbers of microdose hits per exposed micromass, and here the sum of microdoses is then very close or identical to D. As D decreases, the number of microdose hits per exposed micromass is reduced. When the number of microdose hits falls far enough below an average value of 1 per micromass, the dose to each micromass becomes either 0 or the microdose from a single track traversing the micromass, and only a fractional number of micromasses experience a microdose hit.

Because the emphasis is on the number of microdose hits in an exposed system, biological effects observed in an exposed system may be viewed as being triggered by all responses to microdose hits. Hence, it is justified to relate the contribution of individual responses to microdose hits from a given radiation quality to the observed tissue effects. At low values of tissue-absorbed dose D, where defined immediate biological effects such as DNA base changes or strand breaks or even chromosomal aberrations increase in proportion to the number of microdose hits, any type of biological response triggered in an individual micromass adds to the expression of a tissue effect. The probability of a particular response to a microdose hit can be expressed on the basis of biological measurements and using the microdosimetry approach. The sum of all response probabilities per microdose hit over all microdose hits in the exposed system then constitutes the net probability of radiation-induced risk to tissue, as seen in Figure 14 [15, 18, 32].

Of course, this approach replaces the term total energy per unit of exposed mass by the distribution weighted sum of energies delivered by the number of microdose hits in that mass. When for a given absorbed dose the exposed mass increases, the number of exposed micromasses increases accordingly. In consequence, the total energy absorbed by the larger mass is higher, as is the number of microdose hits, despite absorbed dose remaining constant (see Figure 12). In other words, the conventional dose–risk function transforms into a microdose hit–number effectiveness function [51]. This opens new avenues for relating tissue effects, or risk to tissue, to the total number of microdose hits for a given radiation quality in any mass of the exposed system.

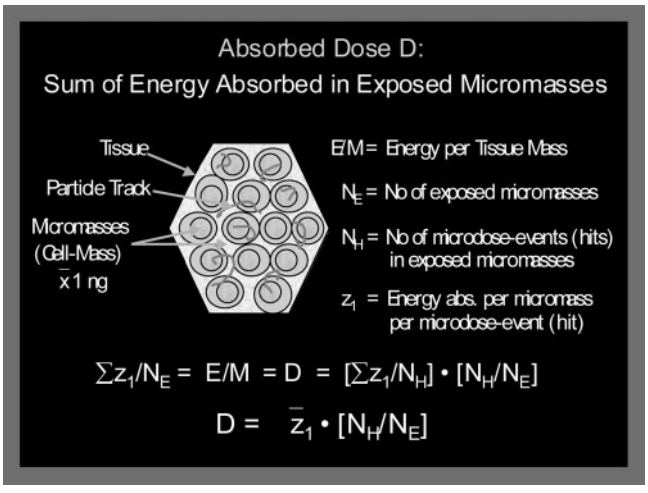


Figure 13. Absorbed dose D is the sum of energy absorbed in exposed micromasses.

Single low dose irradiation

In a single radiation exposure, cell and molecular biological techniques have increasingly yielded a dual pattern of effects in many different cell and tissue systems, as discussed in the earlier section on cellular responses to low doses and dose rates: one effect expressing damage and the other expressing various types of adaptive protection [15, 18, 31, 32]. These responses appear with a delay of hours and may last up to several weeks or months, as was schematically shown in Figure 9. Moreover, the probability of these protective responses

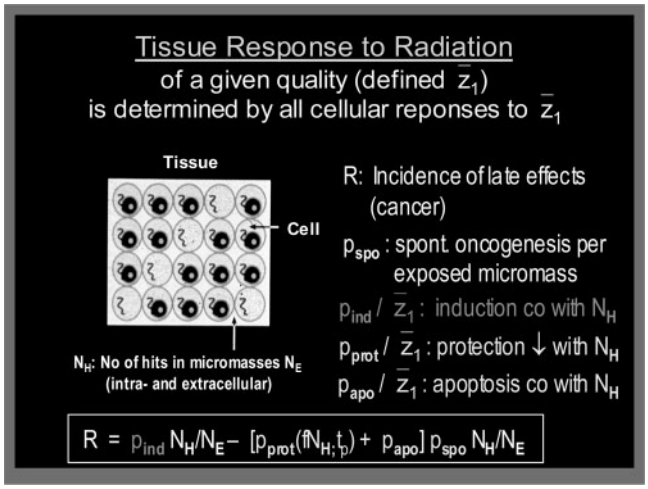


Figure 14. Tissue response to radiation of a given quality derives from all cellular response probabilities per microdose event of size $\bar{z}_1$. In this simplified scheme: p_{spo} is the probability of spontaneous cancer developing from an exposed micromass; p_{ind} is the probability of radiation-induced cancer, which is taken to be constant per microdose event in the low dose range; p_{prot} is the probability of low dose specific protection against cancer, which changes with N_H and t_p; p_{apo} is the probability of radiation-induced apoptosis, which is taken to be constant per microdose event in the low dose range; t_p is the time of duration of protection; N_H is the number of microdose events in exposed micromasses; and N_E is the number of exposed micromasses (see also Figure 13).

varies with cell type and species and except for apoptosis, disappears with absorbed doses above approximately 0.2 Gy, as was indicated schematically in Figure 8. The magnitudes of these responses may result in quite different net risks.

The net risk of damage induction, for instance of oncogenic transformation with lethal outcome, is approximately 10^{-13} to 10^{-14} per microdose hit from low-LET radiation to a potentially oncogenic stem cell [17], and the corresponding probability of a DSB to occur in that cell per hit is approximately 10^{-2}, as was shown in Figure 3 [2]. Thus, the ratio of the two probabilities is approximately 10^{-11} to 10^{-12}. In other words, the probability of a DSB causing a lethal cancer to develop from the affected cell is infinitely small in contrast to the probability of serious DNA damage. Indeed, whereas cancer development from low dose irradiation is barely observable, protective responses are easily measured at such low doses [31, 51, 52]. Because cancer derives mainly from non-radiogenic sources, as shown above, radiation-induced adaptive protection operates mainly against "spontaneous" cancer. Both categories of probabilities, that of damage such as induction of lethal cancer, and that of protection with its mechanisms of defence, DNA repair and damage removal, as discussed above, are the components of the sum of all microdose hit-induced probabilities over all microdose hits in the exposed system, and this sum is equal to the net risk of low dose irradiation. Clearly, the net risk does not necessarily vary with the values of the individual response probabilities per microdose hit, because these individual probabilities express responses that may counteract each other.

The above model predicts that the net cancer risk is lower than would be predicted from a linear no-threshold function. In fact, the model allows for the risk to have a threshold when the probability of oncogenic transformation with lethal outcome per microdose hit is equal to the corresponding probability of protection against cancer development irrespective of the source of the oncogenic transformation. A hormetic negative net risk would ensue if the protection probability per microdose hit outweighs the probability of oncogenic transformation with lethal outcome. Moreover, low doses of ionising radiation may protect against a variety of other diseases such as infections through stimulation of the immune system. Many experimental and epidemiological data on cancer risk and fate of antigen-presenting cells at low doses of ionising radiation agree with the present model approach [32].

Chronic low dose irradiation

With chronic irradiation, microdose hits occur at various time intervals in a defined micromass or in a group of micromasses depending on the dose rate and radiation quality (see Figure 11). Thus, it is crucial to pay attention to the likelihood of interaction between biological responses between two consecutive microdose hits [43]. The probability of any type of biological response to a microdose hit may change as a consequence of responses to a preceding hit in the exposed system. For instance, the time interval may be long enough for the responses to a hit to have subsided, and the affected system may have returned to the homeostatic equilibrium specific for the system before the second event occurs. In this case, the various probabilities following consecutive hits may simply be summed to yield the overall net risk. On the other hand, a microdose hit may meet the target when it is still in response to a preceding hit. In this case, the net risk may be larger or smaller than expected following single microdose hits and this depends on the type and degree of response interactions and modulations between repeat microdose hits. The above model approach accordingly requires some adaptation of components of the equation and this has been a topic of previous work [43].

At low dose rate with an average time interval between two consecutive microdose hits in the target system being long enough for adaptive protection to operate, the net risk may well be negative, *i.e.* beneficial, in that the rate of prevention, repair and/or removal of damage from normal endogenous toxins such as ROS or an improved immune competence outweighs the rate of damage production from repetitive microdose hits or from antigen-presenting cells. A threshold would result if both rates, that of radiation-induced damage and that of prevention of damage from endogenous toxins, would even out during chronic exposure. A dose rate shorter than the optimal time interval for protection to function between consecutive microdose hits would lead to damage accumulation and eventual tissue and even multi-organ failure, depending on the number of vital tissues involved. Indeed, a number of animal experiments conform to such a model and some of these were discussed at this Workshop.

The clinical symptoms and outcome of chronic low dose irradiation in a given organism appear to depend on the values of the various cellular response probabilities per individual microdose hits, on radiation quality that determines the values of the energy deposited per hit,

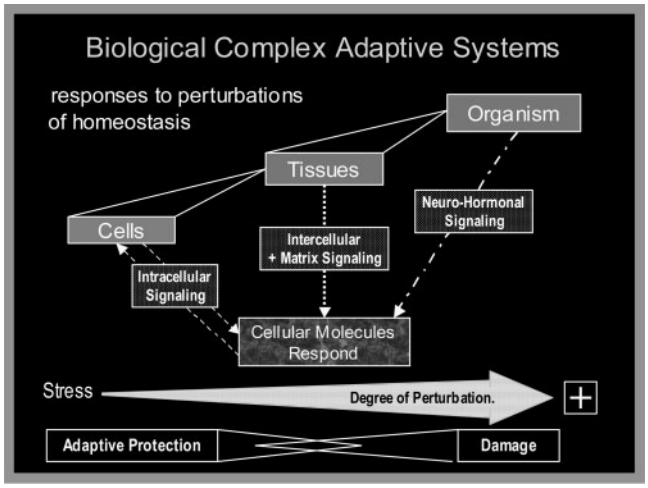


Figure 15. Biological systems such as mammalian organisms maintain homeostatic equilibrium through signalling at all levels of biological organisation. These signals originate from various sites: those that control the entire organism, specific tissue functions, defined cell functions and intracellular functions. The signals always involve cell responses that govern various levels of biological organisation. The signalling serves to secure system integrity and survival in the face of perturbations constantly brought about by exposure to a multitude of endogenous and environmental toxic agents. Small and moderate degrees of perturbations tend to initiate adaptive responses, whereas severe perturbations may lead to system failure and death.

and on dose rate giving the mean time intervals between hits, as discussed above. More work should answer the challenge of understanding tissue and multi-organ failure as a function of the probabilities of the various counteracting responses of cellular systems as components of the tissue system and the entire organism with its largely still elusive signalling networks. A scheme of such networks is shown in Figure 15.

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