

## THE LNT HYPOTHESIS: CAN IT WITHSTAND RECENT DEVELOPMENTS IN MOLECULAR RADIOBIOLOGY AND IN ADAPTIVE PROTECTION MECHANISMS?

### INTRODUCTION

*How cells protect themselves from oxidant stressor agents including radiation is a critical question facing the toxicological community. The role of such adaptive responses and how they affect the nature of the dose-response especially in the low dose zone is likely to play a critical role in the development of more sophisticated assessments of the effects of toxic substances on humans and other species of interest. In this issue of the BELLE Newsletter Drs. Pollycove and Feinendegen provide a detailed assessment of the occurrence of the quantitative and qualitative damages from non-radiation sources, how these compared with exogenous radiation sources and how biological systems adapt to such challenges. The findings also provide an opportunity to re-evaluate the biological plausibility of the Linear No Threshold (LNT) hypothesis that has dominated risk assessment within the chemical and radiation domains. Once the paper of Pollycove and Feinendegen. was received it was sent to several internationally renowned radiation health effects scientists for independent commentary. The paper of Pollycove and Feinendegen is printed immediately below, followed by the expert commentaries and a final statement by Pollycove and Feinendegen.*

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# **RADIATION-INDUCED VERSUS ENDOGENOUS DNA DAMAGE: POSSIBLE EFFECT OF INDUCIBLE PROTECTIVE RESPONSES IN MITIGATING ENDOGENOUS DAMAGE**

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## **ABSTRACT**

Ionizing radiation causes damage to DNA that is apparently proportional to absorbed dose. The incidence of radiation-induced cancer in humans unequivocally rises with the value of absorbed doses above about 300 mGy, in a seemingly linear fashion. Extrapolation of this linear correlation down to zero-dose constitutes the linear-no-threshold (LNT) hypothesis of radiation-induced cancer incidence. The corresponding dose-risk correlation, however, is questionable at doses lower than 300 mGy. Non-radiation-induced DNA damage and, in consequence, oncogenic transformation in non-irradiated cells arises from a variety of sources, mainly from weak endogenous carcinogens such as reactive oxygen species (ROS) as well as from micronutrient deficiencies and environmental toxins. In order to relate the low

probability of radiation-induced cancer to the relatively high incidence of non-radiation carcinogenesis, especially at low-dose irradiation, the quantitative and qualitative differences between the DNA damages from non-radiation and radiation sources need to be addressed and put into context of physiological mechanisms of cellular protection.

This paper summarizes a cooperative approach by the authors to answer the questions on the quantitative and qualitative DNA damages from non-radiation sources, largely endogenous ROS, and following exposure to low doses of ionizing radiation. The analysis relies on published data and justified assumptions and considers the physiological capacity of mammalian cells to protect themselves constantly by preventing and repairing DNA damage. Furthermore, damaged cells are susceptible to removal, for instance, by apoptosis or the immune system. The results suggest that the various forms of non-radiation DNA damage in tissues far outweigh corresponding DNA damage from low-dose radiation exposure at the level of, and well above, background radiation.

These data are examined within the context of low-dose radiation induction of cellular signaling that may stimulate cellular protection systems over hours to weeks against accumulation of DNA damage. The particular focus is the hypothesis that these enhanced and persisting protective responses reduce the steady state level of non-radiation DNA damage, thereby reducing deleterious outcomes such as cancer and aging.

The emerging model urgently needs rigorous experimental testing, since it suggests, importantly, that the LNT hypothesis is invalid for complex adaptive systems such as mammalian organisms.

## **INTRODUCTION**

It is well known that ionizing radiation (IR) interferes with cellular functions at all levels. Cell death and late effects such as malignant tumors may result as a consequence of unrepaired damage to DNA in affected cells. Relatively high values of an absorbed dose ( $D$ ), above about 300 mGy, have been used in most experimental studies for practical reasons. In humans exposed to acute gamma ray doses between 300 mGy and 2 Gy, the risk of cancer in the exposed individuals appears to be proportional to  $D$  (UNSCEAR 1994). For purposes of radiation protection and risk assessment, the proportionality of  $D$  and cancer risk has been assumed to extend down to zero  $D$ . This assumption defines the linear-no-threshold (LNT) hypothesis (ICRP 1991).

Even in non-irradiated cells, DNA damage is continuously being produced, in large part due to reactive oxygen species (ROS) generated by normal oxidative metabolism (Ames 1995; Beckman and Ames 1997; Helbock et al 1998) and augmenting effects from various environmental toxins and deficiencies of micronutrients (Blount et al 1997; Ames 1998; Fenech 1999), which are in part antioxidants or involved in metabolism and repair processes. Normal cells have various mechanisms

for preventing and repairing such damage and removing damaged cells (Potten 1977; Kondo 1993; Wei et al. 1993; Alberts et al. 1994; Friedberg 1995; Hanawalt 1995; Lohman et al. 1995; Jaruga and Dizdaroglu 1996; Wallace 1998; Ohyama and Yamada 1998; Radiation Res. Soc. 1998; Eisen and Hanawalt 1999; Melov et al. 2000; Wood et al. 2001). These mechanisms are here collectively denoted as the DNA damage-control system. At the level of tissues, the immune system physiologically operates also removing damaged cells (Abbas et al. 2000) and, therefore, is here included in the DNA damage control system. Deficiency in this system may cause disease, accelerate aging and increase the probability of malignant transformation and cancer (Cleaver 1968; Wei et al. 1993; Kirkwood and Austad 2000). These components of prevention and repair of DNA damage, and removal of damaged cells physiologically operate at various levels of biological organization, functioning as an efficient network that maintains homeostasis of the organism in the face of constant challenges from potentially toxic doses of various agents from endogenous or environmental sources.

Ionizing radiation also induces ROS. However, with low absorbed doses to cells, in addition to damaging DNA, ionizing radiation has been shown in various species and cell types to activate cellular signaling with stimulation of various components of the DNA damage-control system. Thus, there is direct evidence of low-dose ionizing radiation stimulation of radical detoxification by increased antioxidants (Feinendegen et al. 1987, 1988; Yamaoka 1991; Kojima et al. 1998), prevention of persistent DNA damage, probably by repair (Wolff et al. 1988; Ikushima et al. 1996; Le et al. 1998); and removal of damaged cells, either by apoptosis (Potten 1977; Kondo 1993, 1999; Norimura et al. 1996; Ohyama and Yamada 1998) or by stimulated immune response (James and Makinodan 1990; Makinodan 1992; Anderson 1992; Sakamoto et al. 1997; Hashimoto et al. 1999). These responses have been described in different cell types and constitute mechanisms of adaptation. Most of these protective responses are induced to last for hours to weeks after low, but not high, cell doses (Shadley and Wienke 1989; James and Makinodan 1990; Anderson 1992; UNSCEAR 1994; Feinendegen et al. 1995; Joiner et al. 1996; Feinendegen et al. 1999). At high cell doses damage prevails and adaptive responses are not observed. Single exposures to low doses or low dose rates may thus stimulate the physiological DNA damage control system that helps to reduce the steady state level of non-radiation DNA damage. This non-radiation damage appears to be greater by several orders of magnitude than the DNA damage caused by low-dose ionizing radiation, i.e., below 300 mGy (Feinendegen et al. 1995, 1999; Pollycove and Feinendegen 1999). However, the quantitative and qualitative relationships between the DNA damages from non-radiation and radiation sources remain open questions.

These relationships are crucial to understanding the responses of biological systems to low-dose irradiation.

Existing experimental and epidemiological data on low-dose, low-LET radiation effects support threshold or even hormetic effects, rather than a linear dose response (Feinendegen and Pollycove, 2001; Pollycove and Feinendegen 2001).

The present paper summarizes and extends a cooperative approach to answer these questions during a meeting in Berkeley, California, on June 2 and 3, 1998. The analyses rely on published data and justified assumptions. The particular focus is the experimentally justified hypothesis that low-dose ionizing radiation-induced cellular signaling that over hours to weeks stimulates various mechanisms of physiological control concomitantly reduces the steady state level of non-radiation DNA damage. The model in this paper urgently needs more rigorous experimental testing, in that it suggests, importantly, that the LNT hypothesis may be invalid.

## Quantitative Aspect of DNA Damage

### 1. Ionizing Radiation

The interaction of ionizing radiation with biological systems inevitably results in abundant production of ROS following the ionization of water molecules (von Sonntag 1987; Beckman and Ames 1998). The spacing of these ionizations and resulting ROS depends upon the type and energy of ionizing radiation, with their characteristic particle tracks (ICRU 1983). Electrons such as Compton electrons derived from x- or gamma rays and beta particles produce instantaneous ionizations usually more widely dispersed than those derived from protons, alpha particles, and heavy ions, including atomic recoil nuclei from neutron interaction. The average energy loss through both ionization and excitation per unit length of particle track is conventionally specified by the term linear energy transfer (LET) (ICRU 1980); within certain limits its value allows prediction of the degree of biological effects. However, energy loss along tracks is not uniform; clusters of densely spaced ionizations and resultant ROS occur randomly along tracks, and nearly always at the terminal portions of low-LET radiation such as electrons. The heterogeneity of energy deposition both through particle tracks in tissues and through ionizations and excitations along the individual tracks in cells is particularly noteworthy when one analyzes effects from low doses and low-dose rates (Booz and Feinendegen 1988). Regarding dose rate, the time intervals between consecutive energy deposition events in a defined micromass of tissue are crucial for the evolution of biological effects (Feinendegen et al. 1985; Feinendegen and Pollycove 2001).

Radiation effects in cells are produced predominantly by the energy deposited in them. Nonirradiated cells may also be affected, by intercellular factors transferred in part through gap junctions from high dosed neighboring cells (Nagasawa and Little 1992; Azzam et al. 1998; Seymour and Mothersill 2000). At very low doses, only a fraction of cells in tissues experiences single

track events, i.e., are hit. An average electron track caused by 100 keV x-rays produces a dose of about 1 mGy in the "hit" mammalian cell of 1 nanogram (ng) mass average (NCRP 1979; ICRU 1983; Feinendegen et al. 1994). Such a track creates approximately 200 ionizations, about 80% of which cause ROS, many leading to secondary effects. These include membrane peroxidation and DNA alterations as radiolysis products, here termed oxidative damages (DNA oxidamages) in addition to the less frequent DNA damages from direct ionizations (Wallace 1998). The cellular DNA damage produced by 1 mGy, i.e., 1 hit from low-LET radiation per cell, involves an average of about 2 alterations irrespective of their nature (Ward 1988). This is roughly equivalent to the annual damage per cell in the human body from low-LET background radiation of 1 mGy per year. At this dose rate, each micromass of 1 ng (one cell) is hit on average once per year (Feinendegen and Pollycove 2001). Thus, about 2/365, i.e., about  $5 \times 10^{-3}$  alterations result on average per cell per day in the low-LET exposed body from direct hits and some bystander effects may perhaps occur but are likely negligible at such low doses. A minute fraction of cells hit from natural high-LET exposure add corresponding direct and bystander effects, mainly in the respiratory tract from radon and its daughter nuclides. Lasting functional changes of cellular matrix must be considered but are less likely at low-LET low doses (Weaver et al. 1996).

## 2. Endogenous Metabolism

Mammalian cells are known to carry a steady state level of DNA damage. A few of these alterations are produced by errors during DNA replication but with a very low probability. The low error frequency of  $10^{-10}$  mistakes per base pair each replication cycle is derived from  $10^{-5}$  for initial base pairing,  $10^{-2}$  for proofreading and  $10^{-3}$  due to mismatch repair (Friedberg et al. 1995). Relatively few errors arise also by thermal instability of DNA yielding deamination of cytosine ( $10^2$ /cell/d) and depurination ( $5 \times 10^3$ /cell/d) (Alberts et al. 1994). DNA damage arises mainly from normal oxygen metabolism by way of ROS (Ames et al. 1995; Beckman and Ames 1997; Helbock et al. 1998), as well as by deficiencies of micronutrients (Ames 1998), and other endogenous as well as environmental toxins. The quantity of DNA damage from ROS has been estimated repeatedly. At steady state, the number of DNA oxidamages per cell in rat and human cells from endogenous ROS alone is difficult to measure and reported cellular analyses of steady state numbers per cell range from  $2.4 \times 10^4$  to  $1.2 \times 10^6$  (Kasprzak et al. 1992; Kasprzak et al. 1994; Nakae et al. 1995; Olinski et al. 1996; Jaruga and Dizdaroglu 1996; Nakajima et al. 1996; Olinski et al. 1995; Beckman and Ames 1997; Helbock et al. 1998). The steady state number of  $2.4 \times 10^4$  oxidamages per cell per day is comparable with the recent steady state values reported by Wilson et al. 2001 and the steady state level of  $4 \times 10^3$  apurinic sites in cultured human fibroblasts (Atamna et al. 2000). In the special case of a human lung carcinoma

cell line in culture (A549) a total of as little as 200 DNA oxidamages per cell may represent the steady state situation (Le et al. 1998). For the present discussion, the low value of  $2.4 \times 10^4$  oxidamages per cell in vivo will be considered to be reasonable until more refined data become available. More than 20 different DNA oxidamages from endogenous sources have been identified. Measured half-lives for the elimination of 10 DNA oxidamages in human lymphoblast cells after  $H_2O_2$  treatment range from 8 minutes to about an hour (Jaruga and Dizdaroglu 1996). As discussed below and in Table 3, 10% of endogenous DNA oxidamages are assumed to form single strand breaks (SSB) with a repair half-life of about 5 minutes (Frankenberg-Schwager 1990). This would not significantly change the average half-life of the DNA oxidamages. For the purpose of the present discussion, an average elimination half-time of 25 minutes is taken for the 10 measured oxidamages; the corresponding repair time for these oxidamages is then about  $25 \times 1/\ln 2$ , i.e., about 36 minutes. Taking these oxidamages to be representative contributors to the steady state of  $2.4 \times 10^4$  oxidamages per cell being replaced every 36 minutes, approximately  $10^6$  DNA oxidamages per cell would be produced daily (Table 1), compared with daily turnover of  $2 \times 10^6$  apurinic sites in living human fibroblasts (Atamna et al. 2000). These data do not consider a published lower value (Helbock et al. 1998) that is widely rejected for reasons of error sources that are stated to relate to the urinalysis of guanine derived oxidamages released by cells, enzymatically degraded in the body and excreted in the urine. Accordingly, the probability of a single DNA nucleotide out of a total of  $6 \times 10^9$  per cell being endogenously damaged per day is taken here to be on average about  $10^6/6 \times 10^9$ , i.e.  $1.5 \times 10^{-4}$  based on the conservative assumption used in Table 1.

## 3. Quantitative Relationship Between Endogenous and Radiation DNA Damage

Comparing the number of endogenous DNA alterations to that produced by the above low-LET background radiation results in the surprising ratio of about  $10^6 / (5 \times 10^3) = 2 \times 10^2$ . This huge ratio indicates that the existing complex system that controls DNA damage and assures cellular integrity probably has evolved in response to endogenous rather than to radiation-induced damage (Lindahl 1996).

## Qualitative Aspect of DNA Damage

### 1. Ionizing Radiation

In order to properly compare the consequences of radiation- and non-radiation-induced DNA damage, a qualitative assessment is needed. Experimental observations on particle tracks at various doses of ionizing radiation, as well as their computer analysis, indicate that the spacing of tracks and their ionizations and corresponding initial ROS along them in tissue and a cell is

not uniform (Booz and Feinendegen 1988; Ottolenghi et al. 1995). A majority of these events, depending upon the LET value, occur in clusters. The frequency of such clusters at cellular sensitive sites determines the biological effect (Ward 1988).

Regarding low-LET radiation, about  $2 \times 10^2$  of the damage to DNA occurs as double-strand breaks (DSB) (Ward 1988). DSB are largely caused by the effect of ionization clusters and considered to be a measure of potentially serious detriment in mammalian cells in comparison to single-strand breaks (SSB) and base changes. The latter two are much more efficiently repaired than are DSB and alone are not very lethal (Ward et al. 1985). The above-noted two DNA alterations per cell per mGy of low-LET radiation include roughly 1 base change, 1 SSB, and  $4 \times 10^2$  DSB. Taking again 1 mGy per year low-LET radiation as a whole body dose from background radiation, the probability of background radiation-induced DSB per day is calculated to be about  $2 \times 10^2$  of the about  $5 \times 10^3$  total radiation-induced alterations per average cell per day, or about  $1 \times 10^4$ , per average cell per day.

## 2. Endogenous Metabolism

In contrast to the clustered distribution of ionizations and initial ROS from ionizing radiation, endogenously produced ROS are comparatively widely spaced, although their production is much higher in some cellular compartments, as in mitochondria, than others (Beckman and Ames 1998). Irrespective of origin, the metabolic production rate of ROS in each cell is large. These radicals have different fates; most are scavenged and short lived; many initiate chain reactions on bilayer lipid membranes reaching throughout the cell; biochemical reactions of ROS with macromolecules such as lipids, sugar moieties, proteins and DNA have been measured in cytoplasm and nucleus. The quantification of such ROS reaction products is beset by technical pitfalls largely stemming from the presence of oxygen during the analyses. In vitro measurement of mitochondrial ROS leakage assesses 2 - 3% of the metabolized oxygen to be converted to ROS, which would amount to an average production of  $10^{10}$  cytoplasmic ROS per cell per day (Chance et al. 1979). Recently, this value for in vivo mitochondrial ROS leakage is estimated to be closer to  $10^9$  (Beckman and Ames 1998) (Table 2). With about  $6 \times 10^3$  of the average cell mass being DNA, the assumption that  $10^{-3}$  of the extra-mitochondrial cellular ROS and their various secondary reaction products referred to above induce  $10^6$  DNA oxidamages per cell per day is not unreasonable. This value of  $10^3$  is also consistent with the production of DNA damage by about  $10^2$  of the primary free radicals stochastically generated in the cell by low-LET radiation. Thus, the above selection of steady state alterations with resulting  $10^6$  DNA oxidamages per cell per day appears justified (Table 1). Therefore, on average about  $10^6$  DNA damage events per cell per day may be estimated to occur from ROS alone and without considering the contribution from micronutrient

deficiencies and environmental toxins. The value of  $10^6$  DNA oxidamages is indicated in Table 1.

Even though the probability of non-radiation-induced DNA oxidamages and secondary DNA alterations from them occurring in clusters is extremely small, the numerical value of clustering effects on DNA from such large numbers is considered not to be negligible. As shown in Table 3 and used in Table 5, 10% of DNA damages largely from  $\cdot\text{OH}$ - radicals and secondary ROS products are assumed to form SSB (von Sonntag et al. 1981, 1987).

Other non-radiation sources of DNA damage also contribute to forming SSB. The above assessment of endogenous DNA damage is increased significantly by DNA damage produced by micronutrient deficiencies. Broken chromosomes due to micronutrient deficiencies appear to be induced by the same mechanism as radiation, but are likely to be of far greater significance. Folate deficiency causes massive uracil incorporation (approximately 4 million per cell) into DNA (Blount et al. 1997), which leads to excision repair of the uracils by uracil-DNA glycosylase and apyrimidinic endonuclease, generating transient SSB that could result in a more hazardous DSB if two opposing breaks are formed (Blount et al. 1997, MacGregor et al. 1997, Fenech et al. 1998). Ten percent of the U.S. population was folate deficient at the level at which these chromosome breaks were observed (Ames 1998). Similarly, deficiencies of the vitamins B12 and B6 also can cause uracil incorporation (Ames, unpublished results) and subsequently chromosome breaks (Fenech 1999). Approximately 4% of the population is deficient (less than half of the RDA) in vitamin B12, and about 10% is deficient in vitamin B6 (Ames 1998). Vitamins C and E can help protect against DNA oxidation, and therefore, deficiencies in either of these two vitamins (15% and 20%, respectively) may lead to DNA damage (Ames 1998). Furthermore, deficiencies in iron (7%) and zinc (18%) induce DNA damage and chromosome breaks (Ames 1998). The main source of many of these micronutrients is from fruits and vegetables in the diet. Over 200 epidemiological studies show that the quarter of the population that eats the fewest fruits and vegetables has about twice the cancer rate, for most types of cancer, as the quarter that eats the most (Block et al. 1992). Given that many micronutrient deficiencies act as radiation mimics in causing DNA damage, micronutrient deficiencies and radiation should be compared for perspective. The prevalence of these deficiencies suggests that micronutrient deficiency may be a considerably larger contributor to DNA damage than radiation at low doses.

SSB from whatever source and when occurring within minutes on opposing strands carrying a SSB within 5 base pairs, or a base damage within 3 to 6 base pairs, of each other are here considered to evolve into 1 DSB (Ward 1988; Chaudhry and Weinfeld 1995; Wallace 1998). This simplistic approach does not reflect the enormous complexity of DSB production from probably not homogeneously distributed metabolic oxidative damages of

DNA. Additional simple and complex DSB arise from different enzyme effects on primary DNA oxidamages and SSB produced by them (Schuenemann and Schulte-Frohlinde (1992); Ventur and Schulte-Frohlinde 1993, 1994; Wallace 1998). In this analysis repair half times are taken to be 5 minutes for SSB and 25 minutes for base damage. These time constraints for a neighboring SSB and/or base damage to yield 1 DSB estimate a conservative probability of  $10^{-7}$  for non-radiation caused daily DNA oxidamages to form a DSB. The consequent probable average endogenous daily production of SSB is 0.1/cell/d (Tables 3,5).

### **3. Qualitative Aspects of Non-Radiation-Induced and Radiation DNA Damage**

It follows that under the assumptions given above, the ratio of DSB per average cell per day produced from non-radiation sources to those induced by low-LET background radiation of 1 mGy per year is on the order of at least  $10^{-1}/10^{-4}$ , namely  $10^3$  (Table 4). This ratio is in close agreement with a recent best estimate of 950 (Stewart 1999). Within this calculated relatively large number of non-radiation-induced DSB, the spectrum of biochemical characteristics is expected to include DSB that are similar to those produced by radiation (Wallace 1998). The efficacy of the complex repair mechanisms operating in normal cells in eliminating most of the relatively rare radiation-induced DSB also justifies this appraisal.

The ratio of all DNA alterations produced per average cell per day from non-radiation sources to those produced by background radiation is at least  $2 \times 10^8$ , as discussed. However, DSB are calculated to comprise only  $10^{-7}$  of the endogenous and probably of all non-radiation-induced DNA alterations whereas they comprise  $2 \times 10^{-2}$  of the background radiation-induced DNA alterations. In other words, the probability of DSB per DNA alteration from background radiation is probably close to  $10^5$  times larger than the corresponding probability per alteration from non-radiation source. This conforms to the qualitative difference in DNA damage and in the overall effectiveness in repairing DNA damage from ionizing radiation and non-radiation sources.

## **DNA Damage-control System**

### **1. The Physiological Components**

The physiological DNA damage-control system, as defined above (see introduction) attempts to preserve the genome and is taken to include mechanisms of: (1) damage prevention, mainly by radical detoxification, (2) enzymatic repair at various levels of damage complexity, and (3) removal of damaged cells by apoptosis, or necrosis and globally by immune responses (Potten 1977; Kondo 1993; Wei et al. 1993; Alberts et al. 1994; Hanawalt 1994; Friedberg et al. 1995; Lohman et al. 1995; Jaruga and Dizdaroglu 1996; Ohyama and Yamada

1998; Wallace 1998; Melov et al. 2000; Abbas et al. 2000; Wood et al. 2000). The relative contribution of each component of the DNA damage-control system is estimated in Figure 1. The magnitude of these respective contributions corresponds with presently accepted cell biology data.

ROS detoxification is considered, as discussed above, to allow  $10^3$  of the cytoplasmic cellular ROS to produce  $10^6$  DNA oxidamages per cell per day (Table 1, Figure 1). Various enzymatic DNA repair mechanisms eventually reduce the entirety of non-radiation-caused DNA alterations to persisting alterations by a factor of about  $10^4$ , i.e., to leave only  $10^2$  persistent DNA alterations from endogenous ROS sources alone (Frankenberg-Schwager 1990) (Appendix, Figure 1); these are still subject to removal. In view of the relative rarity of non-radiation-induced DSB compared to total DNA alterations, the inclusion of a reduction factor of about  $10^4$  for repair of DSB (Friedberg, et al. 1995) does not alter this assessment (Appendix).

Regarding radiation-induced DNA alterations, those from DSB constitute a relatively large fraction of about  $2 \times 10^{-2}$ . The various components of the DNA damage control system are assumed to act according to the type of the radiation-induced alteration. As discussed above, 1 mGy of low-LET background radiation causes about  $5 \times 10^{-3}$  total DNA alterations and  $10^{-4}$  DSB per average cell per day. The persistent damage remaining after repair, then, consists of  $10^{-5}$  DSB per average cell per day with only a minute contribution of about  $5 \times 10^{-7}$  persistent damages other than DSB. Thus, the ratio of persistent damage produced per average cell per day from non-radiation sources - being overwhelmingly other than DSB, i.e.,  $>10^2$ , - to the radiation-induced persistent damage - mainly from DSB, i.e.,  $10^{-5}$ , is  $>10^7$  (Appendix, Table 5). This ratio again emphasizes the importance of non-radiation-induced DNA damage, despite the relatively low number of steady state DNA oxidamages assumed for the present discourse.

Finally, cells with persistent DNA alterations, i.e., alterations that either escaped repair or are DNA mutations, may eventually be removed from tissues mainly by immune mechanisms and apoptosis; this also holds for cells predamaged by low doses (UNSCEAR 1994). The persisting DNA alterations in remaining cells are here termed mutations, whether non-radiation- or radiation-induced. The estimated reduction factor of  $10^2$  appears conservative and here appropriate in view of the known immune response to spontaneous and transplanted tumor cells in rodents and humans and the known relationship between immune dysfunction and the development of infections and malignant tumors (Anderson 1992; Makinodan 1992; UNSCEAR 1994; Sakamoto et al. 1997; Hashimoto et al. 1999; Abbas et al. 2000). The above ratio of  $>10^7$  for persistent DNA damages from the non-radiation to radiation sources remains unchanged for the correspondingly produced mutations.

Thus, in this assessment, the DNA damage-control

system reduces the probability of damages only produced by ROS alterations of DNA in normal cells by a factor of  $10^6$  to about 1 mutation per cell per day (Figure 1). With this assumed lower value of the natural average rate of accumulation of mutations, a human cell would acquire about 400 DNA mutations per year, or close to  $3 \times 10^4$  over a period of 70 years. This would amount to a probability of about  $10^{-5}$  average for an individual base pair to change in the human genome by the time old age is attained. However, this estimated  $10^{-5}$  average is low. The gradual accumulation of mutations with age contributes to weakening the DNA damage-control system, thereby allowing a corresponding increase in residual daily mutations.

## ***2. The Effect of Low-Dose of Low-LET Radiation on the Damage Control System***

The various components of the DNA damage-control system may be stimulated by low concentrations of ROS. For instance, the human apurinic/aprimidinic (AP) endonuclease (APE) that plays a central role in repairing DNA oxidamages, may be activated selectively by sublethal levels of a variety of ROS and ROS generators, including ionizing radiation, but not other genotoxicants, such as UV light and alkylating agents (Ramana et al. 1998). Likewise, vitamin-E deficiency, as shown in mouse bone marrow, may induce biochemical reactions that are linked to ROS detoxification (Feinendegen et al. 1987; Hohn-El Karim et al. 1990). Alterations in concentrations of cellular ROS may control enzymes in apoptosis, the expression of various genes, also those that participate in cellular defense, repair and damage removal, and influence the biology of aging (Chandra et al 2000; Finkel and Holbrook 2000). These data suggest that cellular ROS may directly or indirectly produce or suppress DNA alterations depending on ROS concentration. In this context, the system has no way of knowing the source of the ultimate lesion in DNA, be it from ionizing radiation or non-radiation sources.

In view of the direct or indirect low-dose induction of a burst of ROS in the cell per energy deposition event, consecutive signaling effects may at least in part be responsible for the observation on low-dose induced stimulation of various components of the DNA damage control system. As already alluded to above, low-doses of low-LET radiation have been shown in various species and cell types to stimulate the control system at molecular and cellular levels over prolonged periods of time, from hours to weeks: radical detoxification (Feinendegen et al. 1987, 1988; Yamaoka 1991; Kojima et al. 1998); prevention of persistent DNA damage, probably by repair (Wolff et al. 1988; Ikushima et al. 1996; Le et al. 1998); and removal of damaged cells, either by apoptosis (Potten 1977; Norimura et al. 1996; Ohyama and Yamada 1998; Kondo 1993, 1999) or by stimulated immune response (James and Makinodan 1990; Makinodan 1992; Anderson 1992; Sakamoto et al. 1997;

Hashimoto et al. 1999).

With the above assumptions and justified approximations, the ratio of DSB from non-radiation sources to those from low-LET background radiation is calculated to be about  $> 10^3$  (Table 4), and the corresponding ratio of the overall remaining DNA damage to be  $> 10^7$  (Appendix). This assessment may need adjustment by orders of magnitude depending on the individual cellular extent of non-radiation damage to DNA. Nevertheless, the prevalence of non-radiation DNA damage over damage from background radiation is in agreement with the generally accepted high ratio of cancers from these two sources. It follows that the low-dose induction of the DNA damage control system acting over hours to weeks after irradiation obviously affects mainly DNA damage from non-radiation sources, even at considerably higher dose levels than those from background radiation (Table 5).

In order to now introduce the question of the probable net effect following low-dose irradiation, first, low-LET radiation is considered, as it may cause low cell doses even from single hits; second, immediate radiation-induced DNA alterations are known to increase as a linear function of dose; third, the various components of the physiological DNA damage-control system can respond to low cell doses, as referenced above (Figure 2). These responses, except for apoptosis, whenever examined as function of dose show dose-dependence in that they increasingly disappear with doses above about 200 mGy (Feinendegen et al. 1999). In fact, this particular response pattern is in principle very similar to that frequently seen in toxicology and pharmacology and typically expresses the ubiquitous characteristics of complex adaptive systems. As one of many examples: increasing concentrations of penicillin initially stimulate growth of *Staphylococcus* cultures to about 150% of control at about 0.015 units penicillin per ml broth; with higher penicillin concentrations bacterial growth falls steeply and linearly, as expected (Miller et al. 1945). With the evolution of system complexity from prokaryotes to eukaryotes, to mammalian systems, the mechanisms of system adaptation to potentially toxic agents appear to become increasingly convoluted (Gell-Mann 1994).

With the above facts and assumptions the net effect of an increased low-LET background radiation from 1 to 10 mGy per year portrays an interesting scenario, as shown in Figure 2. The increase of the background dose by a factor of 10 reduces the ratio of initial endogenous DNA alterations per average cell per day to those from radiation from  $2 \times 10^8$  to  $2 \times 10^7$ . This rise in background radiation will increase the number of cells hit per day from about  $1/365$  ( $\sim 0.003$ ) to about  $10/365$  ( $\sim 0.03$ ). For the purpose of this discussion, these cells hit daily, 3% of the total number, are each assumed to respond with a 60% enhanced effectiveness of the entire DNA damage-control system, lasting over a period of 10 days. Consequently, 30% of the cells will accordingly be affected every 10 days with an increased total effectiveness of 60% for protective responses. Thus, the average increase in

protective response for all cells will be 0.3 of 60%, i.e., about 20%. This assumption is a first approximation that is consistent with the corresponding finding of a 70% decrease of spontaneous malignant transformations in cell culture that includes the additional responses of cells not hit to signaling from hit cells and to hits in the medium. This decrease in transformation incidence remains constant following exposures to 1, 10, and 100 mGy, i.e., whether each cell received an average of 1, 10, or 100 hits (Azzam et al. 1996; Redpath and Antoniono 1998). The resulting estimate of an overall reduction of fixed DNA damages by 20% at 10 mGy per year is also consistent with reports of health status of populations and corresponding radiobiology studies in high natural background radiation areas of India (Kesavan 1996; Nair et al. 1999; Cheriyan et al. 1999; Jakrishan et al. 1999), Iran (Sohrabi 1990; Mortazawi et al. 2001), and decreased cancer mortality rates observed in populations living in "high radiation background areas" (Kostychenko et al. 1994), the largest cohort of occupational nuclear workers (Matanoski 1991), Mayak plutonium workers (Tokarskaya et al. 1997), and medical cohorts (Miller et al. 1989; Webster 1992; Rossi and Zaider 1997; Sakamoto et al. 1997; Polycove 1994, 1998).

## DISCUSSION

The progressive lifetime accumulation in stem cells of numerous mutations initiated predominantly by endogenous metabolic ROS is implicated in aging, associated degenerative diseases, and cancer (Finkel and Holbrook 2000; Kirkwood and Austad 2000; Rattan 2000; De Pinho 2000). Although the number of DNA alterations per cell per day produced by ROS, their secondary products, micronutrient deficiencies, and environmental toxins remains controversial, the accelerating accumulation of a large number of mutations throughout life is a constraining boundary condition. The low steady state value of 200 DNA oxidamages per cell obtained from culture of a human lung carcinoma cell line (Lee et al. 1998) would correspond to less than  $10^4$  DNA oxidamages per cell per day. Since it is well accepted that DNA repair reduces DNA alterations, other than relatively rare DSB, by a factor of  $10^4$ , a steady state of 200 DNA oxidamages per cell would preclude significant contribution from ROS to accumulation of mutations in these cells. The importance of immune surveillance and apoptosis for removal of persistent unrepaired or misrepaired alterations suggests that the steady state value of DNA oxidamages per cell is not significantly less than  $2.4 \times 10^4$  in mammalian stem cells.

The above assessment of non-radiation- and radiation-induced DNA damage, both quantitatively and qualitatively, reiterates the need for including the level of fixed DNA damages from non-radiation sources when considering the effect of low doses of ionizing radiation. Crucial are:

- 1) The extent of the non-radiation DNA damage appears to far outweigh the DNA damage induced

by low-dose, low-LET radiation, even if the steady state cell burden of oxidamages were an order of magnitude less than assumed. The ratio of DSB from the two sources falls as the square of the fractional reduction of primary DNA oxidamages (Table 5).

- 2) Consequently, low-dose induced stimulation of the physiological DNA damage-control system, be it that of radical detoxification, DNA repair, or cell removal by apoptosis and immune responses, is expected to act predominantly on the non-radiation-induced DNA alterations over hours to weeks after irradiation. This delayed stimulation, except for apoptosis appears limited to low doses for it disappears at high doses. In contrast to low-dose responses, the well-studied DNA repair mechanisms are the apparent main immediate cellular response operating after high doses.

Different from low doses of low-LET radiation, high doses and high dose rates cause DNA damage outweighing non-radiation damage. A single whole body exposure to 5 Gy of gamma radiation given over a period of one minute can be lethal to humans if not properly treated. Yet, this dose produces within about a minute only some  $10^4$  DNA alterations per cell. On the other hand,  $10^6$  DNA oxidamages are taken to be produced from non-radiation sources per day, i.e. about 700 per minute (Figure 1). Thus, during the one minute exposure the ratio of  $10^4$  radiation-induced DNA alterations to about 700 non-radiation DNA oxidamages per cell average is about 15 : 1, with a corresponding DSB ratio of  $3 \times 10^6$  : 1. For comparison, the corresponding ratios are  $5 \times 10^9$  : 1 and  $10^3$  : 1 per cell per day respectively, as they result per cell average from about  $5 \times 10^3$  daily background radiation-induced DNA alterations to  $10^6$  alterations from endogenous sources.

This very large difference of DSB from radiation- and non-radiation-induced DNA damage is, of course, the consequence of the numerical and qualitative difference between the DNA alterations from the two sources, as discussed above. High doses of ionizing radiation not only cause a dose dependent amount of DNA damage, but are also expected to disrupt the homeostatic cell function of the DNA damage-control system, as was shown indirectly for the radical detoxification system (Feinendegen et al. 1995). Obviously, multiple functional defects caused by high doses eventually determine the fate of the cell.

Indeed, the doses absorbed by a given number of individual cells and perhaps corresponding masses of inter-cellular space are of greater importance than absorbed dose to tissue. A tissue effect is the consequence of all cellular responses. With increasing tissue doses, the fraction of cells experiencing energy deposition from a particle track, i.e., being hit, increases to one and eventually all cells experience multiple hits proportional to dose. With low-LET radiation, the individual

hit to cells causes on average a relatively low cell dose, whereas a hit from high-LET radiation causes on average a relatively high cell dose. Thus, a single hit to a cell from 100 keV rays delivers an average cell dose of about 1 mGy, while a hit from a 4 MeV alpha particle delivers an average cell dose of about 300 mGy (ICRU 1983). The biological effects in the hit cells differ accordingly.

In the low-dose region of high-LET radiation, where the fraction of cells hit is below one, non-hit neighboring cells have also shown detriment, for example, chromosomal aberrations, from factors which can be transferred from one cell to another (Nagasawa and Little 1992; Sawant et al. 2001), so-called clastogenic factors causing bystander effects. Such signaling effects may also alter gene expression (Azzam et al. 1998) and need special attention regarding the ratio of detriment to benefit in the exposed tissue. The intercellular signaling also appears to be linked to ROS (Narayanan et al. 1997). Indeed, if toxic agents irrespective of their origin cause DNA damage, at low concentrations they should principally be considered also to stimulate the physiological DNA damage-control system. In the case of clastogenic factors, the relative contributions of the damaging and protecting cellular responses to the toxic agent again are likely to depend on the agent's concentration in the cell. Moreover, though clastogenic factors may persist for years after irradiation (Emerit et al. 1996), statistically significant human epidemiologic studies have shown that low-dose high-LET radiation of the lung decreases the risk of lung cancer (Tokarskaya et al. 1997; Cohen 1995). Although these concentrations and time dependent relationships are currently unknown, the balance between damaging and protecting cellular responses may decide the degree of net damage also from clastogenic factors in intact biological tissues. Effects from the irradiated matrix on cellular functions also need to be considered (Weaver et al. 2000).

In principle, ionizing radiation has been shown to have a dual effect at low cell doses, one of which causes DNA damage and the other stimulates the physiological system that constantly controls the sources and consequences of the level of steady state endogenous DNA damage. At high cell doses the protecting effect, except that for apoptosis, has been observed to disappear. The balance between these two principal effects at various cell dose levels appears crucial for the outcome of tissue response.

## SUMMARY

On the basis of experimental data and justified assumptions, one may state that steady state of non-radiation-induced DNA damage far outweighs DNA damage from low doses of low-LET radiation, both quantitatively and qualitatively. Because tissue effects are predominantly the consequence of individual cellular responses, the doses to micromasses of cellular dimensions, i.e. cell doses, in tissues appear primarily more relevant than the conventional absorbed dose to tissue. Low cell doses, and perhaps also correlated intercellu-

larly operating factors, unequivocally cause a dual effect, as shown in various cell types and species: 1) a rise in DNA damage above background as a function of dose, and 2) a stimulation of the physiological DNA damage control system in terms of ROS detoxification, DNA repair and removal of damaged cells. This stimulation lasts from hours to weeks after irradiation and, except that of apoptosis, vanishes at high cell doses.

The low-dose induced stimulation of the DNA damage-control system appears as a physiological stress response, which also operates on endogenous ROS and non-radiation-induced DNA alterations over prolonged periods of time. Since non-radiation-induced DNA alterations and ensuing damage apparently far exceed corresponding effects from low-dose and low dose rate of low-LET radiation, the radiation-induced adaptive responses are expected to affect predominantly the non-radiation DNA damage for a prolonged period of time after individual cells experience energy deposition events.

The physiological DNA damage-control system, in fact, operates as an antimutagenic biosystem. It follows that gene mutations caused by non-radiation sources are expected to be reduced accordingly by low doses of low-LET radiation. There is reason to assume that this also occurs following low doses of high-LET radiation, where cell doses are high but the fraction of hit cells is low, and intercellularly operating factors have been observed. The line of reasoning and the conclusions of this report suggest targeted experimental approaches to verify them and directly confirm the low-dose induced reduction of the level of non-radiation-induced DNA damage and related incidence of cancer in the low-dose exposed population. As more refined data becomes available, such data can replace the assumptions used and alter the calculations shown in Tables 1-5.

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## REFERENCES

- Abbas A.K., Lichtman A.H., Pober J.S. (2000). Cellular and Molecular Immunology, 4<sup>th</sup> Ed. Saunders Company, Philadelphia.

- Alberts B., Bray D., Lewis J., Raff M., Roberts K., Watson J.D. Eds. (1994). *Molecular Biology of the Cell*, 3<sup>rd</sup> Ed. Garland Pub., New York, New York.
- Ames B.N., Gold L.S., Willet W.C. (1995). The causes and prevention of cancer. *Proc. Natl. Acad. Sci. USA* 92:5258-5265.
- Ames B.N. (1998). Micronutrients prevent cancer and delay aging. *Tox. Letters* 102-103:5-18.
- Anderson R.E. (1992). Effects of low-dose radiation on the immune response. In: *Biological Effects of Low Level Exposures to Chemicals and Radiation*. Ed. E.J. Calabrese. Lewis Pub. Inc., Chelsea, Michigan, pp. 95-112.
- Atamna H., Cheung I., Ames, B.N. (2000). A method for detecting abasic sites in living cells: Age-dependent changes in base excision repair. *Proc. Natl. Acad. Sci. USA* 97:686-691.
- Azzam E.I., de Toledo S.M., Raaphorst G.P., Mitchel R.E.J. (1996). Low-dose ionizing radiation decreases the frequency of neoplastic transformation to a level below the spontaneous rate in C3H 10T1/2 cells. *Rad. Res.* 146:369-373.
- Azzam E.I., de Toledo S.M., Gooding T., Little J.B. (1998). Intercellular communication is involved in the bystander regulation of gene expression in human cells exposed to very low fluence of alpha particles. *Rad. Res.* 150: 497-504.
- Beckman K.D., Ames B.N. (1997). Oxidative decay of DNA. *J. Biol. Chem.* 272:19633-19636.
- Beckman K.D., Ames B.N. (1998). The free radical theory of aging matures. *Physiol. Rev.* 78:547-581.
- Block G., Patterson B., Subar A. (1992). Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* 18:1-29.
- Blount B.C., Mack M.M., Wehr C.M., MacGregor J.T., Hiatt R.A., Wang G., Wickramasinghe S.N., Everson R.B., Ames B.N. (1997). Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc. Natl. Acad. Sci. USA* 94:3290-3295.
- Booz J., Feinendegen L.E. (1988). A microdosimetric understanding of low-dose radiation effects. *Int. J. Radiat. Biol.* 53:13-21.
- Chance B., Sies H., Boveris A. (1979). Hydroperoxide Metabolism in Mammalian Organs. *Physiol. Rev.* 59(3):527-605.
- Chandra J, Samali A, Orrenius S. (2000). Triggering and modulation of apoptosis by oxidative stress. *Free Radic. Biol. Med.* 29(3-4):323-33.
- Chaudhry M.A., Weinfeld M. (1995). The action of *Escherichia coli* endonuclease III on multiply damaged sites in DNA. *J. Mol. Biol.* 249:914-922.
- Cleaver J. (1968). Defective repair replication of DNA in Xeroderma Pigmentosum. *Nature (London)* 218:652-656.
- Cohen B.L. (1995). Test of the linear no-threshold theory of radiation carcinogenesis in the low dose, low dose rate region. *Health Phys* 68:157-174.
- De Pinho R.A. (2000). The age of cancer. *Nature (London)* 408:248-254.
- Eisen J.A., Hanawalt P.C. (1999). A phylogenomic study of DNA repair genes, proteins and processes. *Mut. Res.* 435: 171-213.
- Feinendegen L.E., Booz J., Bond V.P., Sondhaus C.A. (1985). Microdosimetric approach to the analysis of cell responses at low dose and low dose rate. *Rad. Prot. Dosim.* 13: 299-306.
- Feinendegen L.E., Muehlensiepen H., Bond V.P., Sondhaus C.A. (1987). Intracellular stimulation of biochemical control mechanisms by low-dose low-LET irradiation. *Health Phys.* 52:663-669.
- Feinendegen L.E., Bond V.P., Booz J., Muehlensiepen H. (1988). Biochemical and cellular mechanisms of low-dose effects. *Int. J. Radiat. Biol.* 53:23-37.
- Feinendegen L.E., Bond V.P., Booz J. (1994). The quantification of physical events within tissue at low levels of exposure to ionizing radiation. *ICRU-News* 2:9-13.
- Feinendegen L.E., Loken M., Booz J., Muehlensiepen H., Sondhaus C.A., Bond V.P. (1995). Cellular mechanisms of protection and repair induced by radiation exposure and their consequences for cell system responses. *Stem Cells* 13(1):7-20.
- Feinendegen L.E., Bond V.P., Sondhaus C.A., Muehlensiepen H. (1996). Radiation effects induced by low doses in complex tissue and their

- relation to cellular adaptive responses. *Mut. Res.* 358:199-205.
- Feinendegen L.E., Bond V.P., Sondhaus C.A., Altman K.I. (1999). Cellular signal adaptation with damage control at low doses versus the predominance of DNA damage at high doses. *Compt. Rend. Acad. Sci. Paris, Life Sciences* 322:245-251.
- Feinendegen L.E., Pollycove M. (2001). Biological responses to low doses of ionizing radiation: Detriment versus hormesis. Part 1. Dose responses of cells and tissues. *J. Nucl. Med.* 42:17N-27N.
- Fenech M., Aitken C., Rinaldi J. (1998). Folate, vitamin B12, homocysteine status and DNA damage in young Australian adults. *Carcinogenesis* 19:1163-1171.
- Fenech M. (1999). Micronucleus frequency in human lymphocytes is related to plasma vitamin B12 and homocysteine. *Mut. Res.* 428:299-304.
- Finkel T., Holbrook N.J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature (London)* 408: 239-247.
- Frankenberg-Schwager M. (1990). Induction, repair and biological relevance of radiation-induced DNA lesions in eukaryotic cells. *Rad. Environ. Biophys.* 29:273-292.
- Friedberg E.C., Walker G.C., Siede W. (1995). DNA Repair and Mutagenesis. ASM Press, Washington D.C.
- Gell-Mann M. (1994). The Quark and the Jaguar. W.H. Freeman and Company, New York.
- Hanawalt P.C. (1995). DNA repair comes of age. *Mut. Res.* 336(2):101-113.
- Hashimoto S., Shirato H., Hosokawa M., Nishioka T., Kuramitsu Y., Matsushita K., Kobayashi M., Miyasaka K. (1999). The suppression of metastases and the change in host immune response after low-dose total-body irradiation in tumor-bearing rats. *Rad. Res.* 151:717-724.
- Helbock H.J., Beckman K.B., Shigenaga M.K., Walter P.B., Woodall A.A., Yeo H.C., Ames B.N. (1998). DNA oxidation matters: The HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proc. Natl. Acad. Sci. USA* 96:288-293.
- Hohn-El-Karim K., Muehlensiepen H., Altman K.I., Feinendegen L.E. (1990). Modification of effects of radiation on thymidine kinase. *Intern. J. Rad. Biol.* 58:97-110.
- ICRP (International Commission on Radiological Protection). (1991). Recommendations of the International Commission on Radiological Protection. ICRP Publication 60. *Annals of the ICRP* 21 (1-3), Pergamon Press Oxford.
- ICRU (International Commission on Radiation Units and Measurements). (1980). Radiation Quantities and Units. ICRU, Bethesda, MD, USA, Report 33.
- ICRU (International Commission on Radiation Units and Measurements). (1983). Microdosimetry. ICRU, Bethesda, MD, USA, Report 36.
- Ikushima T., Aritomi H., Morisita J. (1996). Radioadaptive response: Efficient repair of radiation-induced DNA damage in adapted cells. *Mut. Res.* 358:193-198.
- James S.J., Makinodan T. (1990). T-cell potentiation by low dose ionizing radiation: possible mechanisms. *Health Physics* 59:29-34.
- Jaruga P., Dizdaroglu M. (1996). Repair of products of oxidative DNA base damage in human cells. *Nucleic Acid Res.* 24:1389-1394.
- Joiner M.C., Larnbin P., Malaise E.P., Robson T., Arrand J.E., Skov K.A., Marples B. (1996). Hypersensitivity to very low single radiation doses: Its relationship to the adaptive response and induced radioresistance. *Mut. Res.* 358:171-183.
- Joiner M.C., Lambin P., Marples B. (1999). Adaptive response and induced resistance. *Compt. Rend. Acad. Sci. Paris, Life Sciences* 322:167-75.
- Kasprzak K.S., Diwan B.A., Rice J.M., Misra M., Olinski R., Dizdaroglu M. (1992). Nickel (II)-mediated oxidative DNA base damage in rat renal chromatin in vivo. Possible relevance to nickel(II) carcinogenesis. *Chem. Res. Toxicol.* 5:809-815.
- Kasprzak K.S., Zastawny T.H., North S.L., Riggs C.W., Diwan B.A., J.M., Dizdaroglu M. (1994). Oxidative DNA base damage in renal, hepatic and pulmonary chromatin of rats after intraperitoneal injection of cobalt acetate. *Chem. Res. Toxicol.* 7:329-335.

- Kesavan P.C. (1996). Indian research on high levels of natural radiation: pertinent observations for further studies. In: High levels of Natural Radiation. Radiation Dose and Health Effects. Eds. L. Wei, T. Sugahara, Z. Tao. Elsevier, Amsterdam, Beijing, China, 1996, 111-117
- Kirkwood T.B.L., Austad S.N. (2000). Why do we age? *Nature* (London) 408:233-238.
- Kojima S., Matsuki O., Nomura T., Kubodera A., Honda Y., Honda S., Tanooka H., Wakasugi H., Yamaoka K. (1998). Induction of mRNAs for glutathione synthesis-related proteins in the mouse liver by low doses of g-rays. *Biochim. Biophys. Acta* 1381:312-318.
- Kondo S. (1999). Evidence that there are threshold effects in risk of radiation. *J. Nucl. Sci. Technol.* 36:1-9.
- Kondo S. (1993). Health Effects of Low Level Radiation. Kinki Univ.Press, Osaka, Japan; Medical Physics Publishing, Madison, WI.
- Kostyuchenko V.A., Krestina L.Y. (1994). Long term radiation effects in the population evacuated from the East Urals radioactive trace area. *The Sci. Total Environ.* 142:118-125.
- Le X.C., Xing J.Z., Lee J., Leadon S.A., Weinfeld M. (1998). Inducible repair of thymine glycol detected by an ultrasensitive assay for DNA damage. *Science* 280:1066-1069.
- Lindahl T. (1996). Endogenous damage to DNA. *Phil. Trans. R. Soc. Lond. B.*
- Lohman P.H.M., Cox R., Chadwick K.H. (1995). Role of molecular biology in radiation biology. *Int. J. Rad. Biol.* 68:331-340.
- MacGregor J.T., Wehr C.M., Hiatt R.A., Peters B., Tucker J.D., Langlois R.G., Jacob R.A., Jensen R.H., Yager J.W., Shigenaga M.K., Frei B., Eynon B.P., Ames B.N. (1997). Spontaneous genetic damage in man: evaluation of interindividual variability, relationship among markers of damage, and influence of nutritional status. *Mut. Res.* 377:125-135.
- Makinodan T. (1999). Cellular and subcellular alteration in immune cells induced by chronic, intermittent exposure in vivo to very low dose of ionizing radiation (ldr) and its ameliorating effects on progression of autoimmune disease and mammary tumor growth. In: Low-Dose Irradiation and Biological Defense Mechanisms. Ed.: Sugahara T., Sagan L.A., Aoyama T.; Excerpta Medica; Amsterdam, London, New York, Tokyo. pp. 233-237.
- Matanoski G.M. (1991). Health effects of low level radiation in shipyard workers final report. Report No. DOE DE-AC02-79 EV 10095. Washington: US Department of Energy.
- Melov S., Ravenscroft J., Malik S., Gill M.S., Walker D.W., Clayton P.E., Wallace D.C., Malfroy B., Doctrow S.R., Lithgow G.J. (2000). Extension of life span with superoxide dismutase/catalase mimetics. *Science* 289:1567-1569.
- Miller A.B., Howe G.R., Sherman G.J., Lindsay J.P., Yaffe M.J., Dinner P.J., Risch H.A., Preston D.L. Mortality from breast cancer after irradiation during fluoroscopic examination in patients being treated for tuberculosis. (1989). *N. Engl. J. Med.* 321:1285-1289.
- Miller W.S., Green C.A., Kitchen H. (1945). Byphasic action of penicillin and other sulphonamide similarity. *Nature* 155:210-211.
- Mortazawi S.M.J., Ghiassi-nejad, Beitollahi M., Cameron J.R., Niroomand-rad A., Karam P.A., Assaie R., Heidary A., Varzegar R., Zakeri F., Jafari M. (In Press). Very high background radiation areas of Ramsar, Iran: Preliminary biological studies and possible implications. *Health Phys.*
- Nagasawa H., Little J.B. (1992). Induction of sister chromatid exchanges by extremely low doses of alpha-particles. *Cancer Res.* 52:6394-6396.
- Nakae D., Mizurnoto Y., Kobayashi E., Noguchi O., Konishi Y. (1995). Improved genomic/nuclear DNA extraction for 8-hydroxydeoxyguanosine analysis of small amounts of rat liver tissue. *Cancer Lett.* 97:233-239.
- Nakajima M., Takeuchi T., Morimoto K. (1996). Determination of 8-hydroxydeoxyguanosine in human cells under oxygen-free conditions. *Carcinogenesis* 17:787-791.
- Narayanan P.K., Goodwin E.H., Lelmert B.E. (1997). Alpha particles initiate biological production of superoxide anions and hydrogen peroxide in human cells. *Cancer Res.* 57:3963-3971.

- NCRP (National Council on Radiation Protection). (1979). Tritium and Other Radionuclide Labelled Organic Compounds Incorporated in Genetic Materials. NCRP, Bethesda MD, Publication 63.
- Norimura T., Nomoto S., Katsuki M., Gondo, Y., Kondo S. (1996). p 53 dependent apoptosis suppresses radiation teratogenesis. *Nature Med.* 2:577-580.
- Ohyama H., Yamada T. (1998). Radiation-induced apoptosis: a review. In: Apoptosis, its Roles and Mechanisms. Ed.: Yamada T., Hashimoto Y. Business Center for Academic Societies Japan, Tokyo. pp. 141-186.
- Olinski R., Zastawny T.H., Foksinski M., Barecki A., Dizdaroglu M. (1995). DNA base modifications and antioxidant enzyme activities in human benign prostatic hyperplasia. *Free Rad. Biol. Med.* 18:807-813.
- Olinski R., Zastawny T.H., Foksinski M., Windorbska W., Jaruga P., Dizdaroglu M.. (1996). DNA base damage in lymphocytes of cancer patients undergoing radiation therapy. *Cancer Lett.* 106:207-215.
- Ottolenghi A., Merzagora M., Tallone L., Durante M., Paretzke H.G., Wilson W.E. (1995). The quality of DNA double-strand breaks: a Monte Carlo simulation of the end-structure of strand breaks produced by protons and alpha particles. *Rad. Environ. Biophys.* 34(4):239-244.
- Pollycove M. (1994). Positive health effects of low level radiation in human populations. In: Biological Effects of Low Level Exposures: Dose-Response Relationships. Ed. E.J. Calabrese. Lewis Pub. Inc., Chelsea, Michigan. pp. 171-187.
- Pollycove M. (1998). Nonlinearity of radiation health effects. *Env. Health Perspect.* 106:363-368.
- Pollycove M., Feinendegen L.E. (1999). Molecular biology, epidemiology, and the demise of the linear no-threshold (LNT) hypothesis. *Compt. Rend. Acad. Sci. Paris, Life Sciences* 322:197-204.
- Pollycove M., Feinendegen L.E. (2001). Biological responses to low doses of ionizing radiation: Dertriment versus hormesis. Part 2. Dose responses of organisms. *J. Nucl. Med.* 42: 26N-37N.
- Potten C.S. (1977). Extreme sensitivity of some intestinal crypt cells to X and  $\gamma$  irradiation. *Nature* 269:518-521.
- Radiation Research Society (USA). (1998). Program Abstracts, forty-sixth annual meeting in Louisville, Kentucky. Rad. Res. Soc., Oak Brook, IL 60523, USA, 1998
- Ramana C.V., Boldogh I., Izumi T., Mira S. (1998). Activation of apurinic/aprimidinic endonuclease in human cells by reactive oxygen species and its correlation with their adaptive response to genotoxicity of free radicals. *Proc. Natl. Acad. Sci. USA* 95:5061-5066.
- Rattan S.I.S. (2000). Biogerontology: the next step. *Annals New York Acad. Sci.* 908:282-290.
- Redpath J. L., Antoniono R. J. (1998). Introduction of an adaptive response against spontaneous neoplastic transformation in vitro by low-dose gamma radiation. *Rad. Res.* 149:517-520.
- Rossi H.H., Zaider M. (1997). Radiogenic lung cancer: the effects of low doses of low linear energy transfer (LET) radiation. *Rad. Environ. Biophys.* 36:85-88.
- Sakamoto, K., Myojin M., Hosoi Y., Ogawa Y., Nemoto, K., Takai Y., Kakuto, Y., Yamada S., Watabe N. (1997). Fundamental and clinical studies on cancer control with total or upper half body irradiation. *J. Jpn. Soc. Ther. Radiol. Oncol.* 9:161-175.
- Sawant S.G., Randers-Pehrson G., Metting N.F., Hall E.J. (2001). Adaptive response and the bystander effect induced by radiation in C3H 10T(1/2) cells in culture. *Rad. Res.* 165:177-180.
- Schuenemann S., Schulte-Frohlinde D. (1992). Survival of phage M13 with uracils on one or both DNA strands. *Mol. Gen. Genet.* 233:348-354.
- Seymour C.B., Mothersill C. (2000). Relative contribution of bystander and targeted cell killing to the low-dose region of the radiation dose-response curve. *Rad. Res.* 153(5 Pt 1):508-511.
- Shadley J.D., Wienke, J.K. (1989). Induction of the adaptive response by X-rays is dependent on radiation intensity. *Int. J. Radiat. Biol.* 56:107-118.
- Stewart R. D. (1999). On the complexity of the DNA damages created by endogenous processes. *Rad. Res.* 152:1101-1102.
- Tokarskaya Z.B., Okladnikova N.D., Belyaeva Z.D., Drozhko E.G. (1997). Multifactorial analysis of

- lung cancer dose-response relationships for workers at the Mayak Nuclear Enterprise. *Health Phys* 73:899-905.
- UNSCEAR. (1994). Sources and Effects of Ionizing Radiation. Annex B: Adaptive Responses to Radiation in Cells and Organisms. United Nations, New York, N.Y. pp. 185-272
- von Sonntag C., Hagen U., Schoen-Bopp A., Schulte-Frohlinde D. (1981). Radiation-induced strand breaks in DNA: chemical and enzymatic analysis of end groups and mechanistic aspects. *Adv. Rad. Biol.* 9:109-142.
- von Sonntag C. (1987). The Chemical Basis for Radiation Biology. Taylor & Francis, London, Philadelphia, New York.
- Ventur Y., Schulte-Frohlinde D. (1993). Does enzymatic conversion of DNA single strand damage into double strand breaks contribute to biological inactivation of gamma-irradiated plasmid DNA? *Intern. J. Rad. Biol.* 63:167-171.
- Ventur Y., Schulte-Frohlinde D. (1994). Biological inactivation of pBr322 plasmid DNA by enzyme- and radiation-induced single strand damage under various conditions. *Mut. Res., DNA Repair* 315:65-74.
- Wakasugi H., Yamaoka K. (1998). Induction of mRNAs for glutathione synthesis-related proteins in the mouse liver by low doses of g-rays. *Biochim. Biophys. Acta* 1381:312-318.
- Wallace S.S. (1998). Enzymatic processing of radiation-induced free radical damage in DNA. *Rad. Res.* 150(5 Suppl)150:60-79.
- Ward J.F. (1988). DNA damage produced by ionizing radiation in mammalian cells: Identities, mechanisms of formation, and repairability. *Prog. Nucleic Acid Res. Mol. Biol.* 35:95-125.
- Ward J.F, Blakely W.J., Joner E.I. (1985). Mammalian cells are not killed by DNA single-strand breaks caused by hydroxyl radicals from hydrogen peroxide. *Rad. Res.* 103:383-392.
- Weaver V.M., Fischer A.H., Peterson O.W., Bissell M.J. (1996). The importance of the microenvironment in breast cancer progression: recapitulation of mammary tumorigenesis using a three-dimensional culture assay. *Biochem. Cell Biol.* 74(6): 833-851.
- Webster E.W. (1992). Dose risk in diagnostic radiology: How big? How little? Lauriston S. Taylor lectures in radiation protection and measurements. National Council on Radiation Protection and Measurements, Bethesda, MD 20814, USA.
- Wei Q., Matanoski G.M., Farmer E.R., Hedayati M.A., Grossman L. (1993). DNA repair and aging in basal cell carcinoma: A molecular epidemiologic study. *Proc. Natl. Acad. Sci. USA* 90:1614-1618.
- Wilson III D.M., Barsky D. (2001). The major human abasic endonuclease formation, consequences and repair of abasic lesions in DNA. *Mut. Res.* 40083:1-25.
- Wolff S., Afzal V., Wienke J.K., Olivieri G., Michaeli A. (1988). Human lymphocytes exposed to low doses of ionizing radiations become refractory to high doses of radiation as well as to chemical mutagens that induce double-strand breaks in DNA. *Int. J. Radiat. Biol.* 53(1):39-49.
- Wood R.D., Mitchell M., Sgouros J., Lindahl T. (2001). Human DNA repair genes. *Science* 291:1284-1289.
- Yamaoka K. (1991). Increased SOD activities and decreased lipid peroxide in rat organs induced by low X-irradiation. *Free Radical Biol Med* 11:3-7.

## Endogenous DNA Oxidative Adducts (OX<sub>E</sub>)/cell/d

Assume:

Steady state OX<sub>E</sub>/cell = 2.4 x 10<sup>4</sup> [1]

DNA repair T<sub>1/2</sub> = 25 min (average) [2]

Turnover Time = T<sub>1/2</sub>/ln2 = 25 min/ 0.693 = 36 min

Then,

OX<sub>E</sub>/cell/d = 2.4 x 10<sup>4</sup> x 24 x 60 min / 36 min

**OX<sub>E</sub>/cell/d ~ 10<sup>6</sup>**

Assume:

10<sup>4</sup> of ROS and their secondary reaction products form OX<sub>E</sub> [3]

Then,

OX<sub>E</sub>/cell/d = 10<sup>9</sup> [3,4] x 10<sup>-3</sup>

**OX<sub>E</sub>/cell/d ~ 10<sup>6</sup>**

Table 1. <sup>1</sup>Helbock, et al. 1998; <sup>2</sup>Jaruga, Dizdaroglu 1996; <sup>3</sup>See text: Qualitative Aspect of DNA Damage: 2. *Endogenous Metabolism*; <sup>4</sup>Table 2

## Reactive Oxygen Species (ROS) cell/d

Assume:

2.5x10<sup>-3</sup> metabolized O<sub>2</sub> leaked as ROS in 10<sup>14</sup> cells [1]

2.5x10<sup>3</sup> kcal/d average energy

10<sup>14</sup> cells whole body

1L O<sub>2</sub> = 4.8 kcal

O<sub>2</sub> density = 1.429 g/L

Then,

O<sub>2</sub> metabolized ≈ 1.429 g/L x 2.5 x 10<sup>3</sup> kcal/d / 4.8 kcal/L

≈ 745 g/d . 2.8 x 10<sup>25</sup> mitochondrial •O<sup>-</sup>/d

ROS/cell/d ≈ 2.5 x 10<sup>-3</sup> x 2.8 x 10<sup>25</sup> x 10<sup>-14</sup> ≈ 7.0 x 10<sup>8</sup>

ROS/cell/d ≈ 10<sup>9</sup>

Table 2. <sup>1</sup>Beckman, Ames (1998)

## Endogenous Double Strand Breaks (DSB<sub>E</sub>)/cell/d

For conservative argument, only the biophysical approach to double strand break formation is considered. Enzyme induced DSB<sub>E</sub>s are referred to in the text.

Assume-

Only sugar oxidative adducts produce single strand breaks (SSB).

Oxidative adducts are produced predominantly by •OH:

SSB repair  $T_{1/2} = 5 \text{ min}^{[1]}$  vs 25 min average  $T_{1/2}$  for OX<sub>E</sub> repair.<sup>[2]</sup>

- 15% of adducts are sugars
- 70% of sugar adducts produce SSB
- So that - 10% of oxidative adducts form SSB

$$\text{Steady state SSB/cell} \approx 5/25 \times 0.1 \text{ steady state OX}_E/\text{cell} \approx 0.02 \times 2.4 \times 10^4 \cdot 5 \times 10^2$$

And,

Let P be the probability of an OX<sub>E</sub> to produce a DSB<sub>E</sub> by forming a SSB<sub>E</sub> on any of the 11 bases B<sub>X±5</sub> that oppose SSB<sub>X</sub> of base pair X<sup>[3]</sup>

Then,

P of producing a

$$\begin{aligned} \text{DSB}_E/\text{OX}_E &\approx 0.1 \times 5 \times 10^2 \times 11 \text{ bases} / 6 \times 10^9 \text{ bases} \\ &\approx 10^{-7} \end{aligned}$$

And,

$$\begin{aligned} \text{DSBE/cell/d} &\approx 10^{-7} \text{ DSB}_E/\text{OX}_E \times 10^6 \text{ OX}_E/\text{cell/d} \\ \mathbf{DSBE/cell/d} &\mathbf{\approx 0.1} \end{aligned}$$

Table 3. <sup>1</sup>Frankenberg-Schwager 1990; <sup>2</sup>Jaruga, Dizderoglu 1996;

<sup>3</sup>Ward 1998; Chaudrv. Weinfeld 1985; Wallace 1988

## Radiation vs Endogenous Double Strand Breaks (DSB<sub>R</sub> vs DSB<sub>E</sub>)/cell/d

Background whole body low LET radiation (BG)  $\approx 1 \text{ mGy/y} \approx 2.7 \times 10^{-3} \text{ mGy/d}$

$$\text{DSB}_R/1 \text{ mGy /cell} \approx 4 \times 10^{-2} \text{ [1]}$$

$$\text{BG DSB}_R/\text{cell/d} \approx 4 \times 10^{-2}/\text{mGy} \times 2.7 \times 10^{-3} \text{ mGy/d} \approx 10^{-4} \quad \text{DSB}_E/\text{cell/d} \approx 0.1$$

$$\mathbf{BG \text{ DSB}_R/10^4 \text{ cells/d} \approx 1}$$

$$\mathbf{DSB}_E/10^4 \text{ cells/d} \approx 1 \times 10^3$$

Table 4. <sup>1</sup>Ward 1988. For comparison of radiation-induced and endogenous total and persistent DNA alterations see Tables 5 and 6.

### DNA Alterations/Average Cell/Day

Assumptions	DNA Alterations	Endogenous (E) (mainly metab. ROS)		Radiation-Induced (R) (mainly ROS)		RATIO E/R	
		Initial	After Repair	Initial	After Repair	Initial	After Repair
$2.4 \times 10^4$ Steady State DNA Alterations  1 mGy/y Low LET BG	Total	$1 \times 10^6$	$1 \times 10^2$	$5 \times 10^{-3}$	$1 \times 10^{-5}$	$2 \times 10^8$	$1 \times 10^7$
	DSB	$1 \times 10^{-1}$	$1 \times 10^{-2}$	$1 \times 10^{-4}$	$1 \times 10^{-5}$	$10^3$	$10^3$
$2.4 \times 10^4$ Steady State DNA Alterations  10 mGy/y Low LET BG	Total	$1 \times 10^6$	$1 \times 10^2$	$5 \times 10^{-2}$	$1 \times 10^{-4}$	$2 \times 10^7$	$1 \times 10^6$
	DSB	$1 \times 10^{-1}$	$1 \times 10^{-2}$	$1 \times 10^{-3}$	$1 \times 10^{-4}$	$10^2$	$10^2$
$2.4 \times 10^3$ Steady State DNA Alterations  1 mGy/y Low LET BG	Total	$1 \times 10^5$	10	$5 \times 10^{-3}$	$1 \times 10^{-5}$	$2 \times 10^7$	$1 \times 10^6$
	DSB	$1 \times 10^{-3}$	$1 \times 10^{-4}$	$1 \times 10^{-4}$	$1 \times 10^{-5}$	10	10

Table 5. Quantitative and qualitative aspects of assumed  $2.4 \times 10^4$  steady state endogenous metabolic DNA alterations per cell and 1 mGy/y low LET background (BG) radiation-induced DNA alterations per average cell per day. Assumed repair error of DSB and other DNA alterations are  $10^{-1}$  and  $10^{-4}$  respectively. These estimates of endogenous and radiation-induced DNA alterations are compared with corresponding estimates assuming one magnitude higher background radiation and one magnitude lower steady state metabolic DNA alterations. Estimates are derived from published experimental data (Tables 1, 3, 4, and Appendix).

## Appendix

### ESTIMATE OF TOTAL AND PERSISTENT CELLULAR DNA ALTERATIONS: LOW-LET RADIATION INDUCED ALTERATIONS ( $A_R$ ) AND ENDOGENOUS ALTERATIONS ( $A_E$ )

RADIATION INDUCED ALTERATIONS ( $A_R$ ) PER CELL PER DAY	
Total $A_R$ [ $TA_R$ ] per cell per mGy with double strand breaks [DSB] and all other alterations [OA] <sup>(1)</sup> :	$TA_R / \text{c/mGy} \approx 2 (0.96 \text{ OA} + 0.04 \text{ DSB})$
With low-LET background radiation [BG] whole body of 1mGy/yr	
- aver. BG per cell per year [BG/c/yr] <sup>(2)</sup> :	$BG/\text{c/yr} \approx 1 \text{ mGy}$
- aver. BG per cell per day [BG/c/d]:	$BG/\text{c/d} \approx 1 \text{ mGy}/365\text{d} = 2.7 \times 10^{-3} \text{ mGy}$
Persistent $TA_R$ per cell from BG/c/d after DNA repair [ $PTA_R$ BG/c/d] assuming repair accuracy [ $RA_R$ ] of $10^{-1}$ for DSB and $10^{-4}$ for all other DNA alterations <sup>(3,4)</sup> :	$PTA_R \text{ BG/c/d} \approx BG/\text{c/d} \times TA_R/\text{mGy} \times RA_R$ $\approx 2.7 \times 10^{-3} \times 2 (0.96 \times 10^{-4} + 0.04 \times 10^{-1})$ $\approx 5 \times 10^{-3} \times 10^{-4} + 5 \times 10^{-3} \times 4 \times 10^{-2} \times 10^{-1}$ $\approx 5 \times 10^{-7} + 2 \times 10^{-5}$ $\approx 10^{-5}$
Thus, persistent $TA_R$ per $10^5$ cells per day from BG/c/d after DNA repair [ $PTA_R$ BG/ $10^5$ c/d]	<b><math>PTA_R \text{ BG}/10^5 \text{ c/d} \approx 1</math> (mainly from DSB)</b>
1) Ward, 1998; (2) ICRU 1983 ; (3) Friedberg et al. 1994; (4) Frankenberg-Schwager 1990.	

## ENDOGENOUS ALTERATIONS ( $A_E$ ) PER CELL PER DAY

Persistent total  $A_E$  per cell per day  
 [PTA $_E$ /c/d] after DNA repair include  
 base pair error replication [PA $_{BPR}$ /c/d]  
 various thermal reactions [PA $_T$ /c/d]  
 and endogenous oxidant attacks [PA $_{OXE}$ /c/d]:  

$$PTA_E/c/d = PA_{BPR}/c/d + PA_T/c/d + PA_{OXE}/c/d$$

With  $3 \times 10^9$  base pairs replicated  
 per cell cycle and  $10^2$  cell replications  
 in 70 years ( $2.56 \times 10^4$  days)<sup>(1)</sup>,  
 total average base replication  
 per cell per day [TBR/c/d]:

$$TBR/c/d = 3 \times 10^9 \times 10^2 / 2.56 \times 10^4 d = 10^7$$

and assuming replication accuracy  
 [RA $_{BRP}$ ] of  $10^{-10}$  <sup>(2)</sup>:

$$PA_{BPR}/c/d = TBR/c/d \times RA_{BRP} = 10^7 \times 10^{-10} = 10^{-3}$$

With  $5 \times 10^3$  total alterations from  
 various thermal reactions (depurination,  
 deamination) per cell per day  
 [TA $_T$ /c/d] <sup>(1)</sup> and assuming  
 repair accuracy [RA $_T$ ] of  $10^{-4}$  <sup>(2)</sup>:

$$PA_T/c/d = TA_T/c/d \times RA_T = 5 \times 10^3 \times 10^{-4} = 5 \times 10^{-1}$$

With  $\leq 10^6$  total alterations from  
 endogenous oxidant attacks per cell  
 per day [TA $_{OXE}$ /c/d] <sup>(3)</sup> including  
 $\sim 10^{-1}$  DSB <sup>(4)</sup> and assuming repair  
 accuracy [RA $_{OXE}$ ] of  $10^{-1}$  for DSB  
 and  $10^{-4}$  for other DNA alterations <sup>(2,5)</sup>:

$$PA_{OXE}/c/d = TA_{OXE}/c/d \times RA_{OXE} = 10^6 \times 10^{-4} + 10^{-1} \times 10^{-1} = 10^2$$

Hence:

$$PTA_E/c/d = PA_{BPR}/c/d + PA_T/c/d + PA_{OXE}/c/d = 10^{-3} + 5 \times 10^{-1} + 10^2 = 10^2$$

Thus persistent PTA $_E$   
 per  $10^5$  cells per day  
 after DNA repair [PTA $_E$ /10 $^5$ c/d]:

$$PTA_E/10^5c/d = 10^7 \text{ (with } 10^3 \text{ from DSB)}$$

(1) Alberts et al. 1994; (2) Friedberg et al. 1994; (3) see Table 1; (4) see Table 3; (5) Frankenberg-Schwager 1990

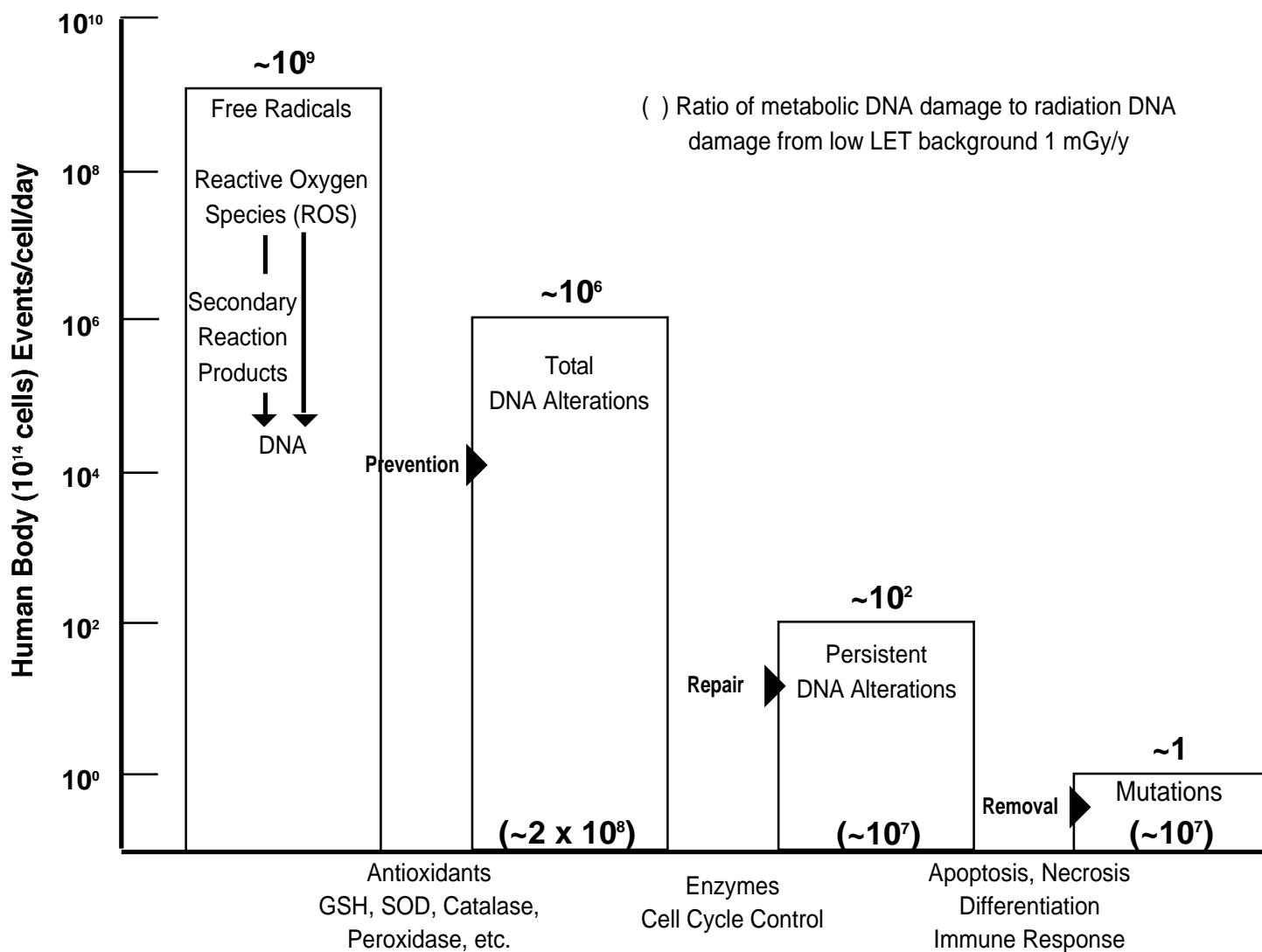
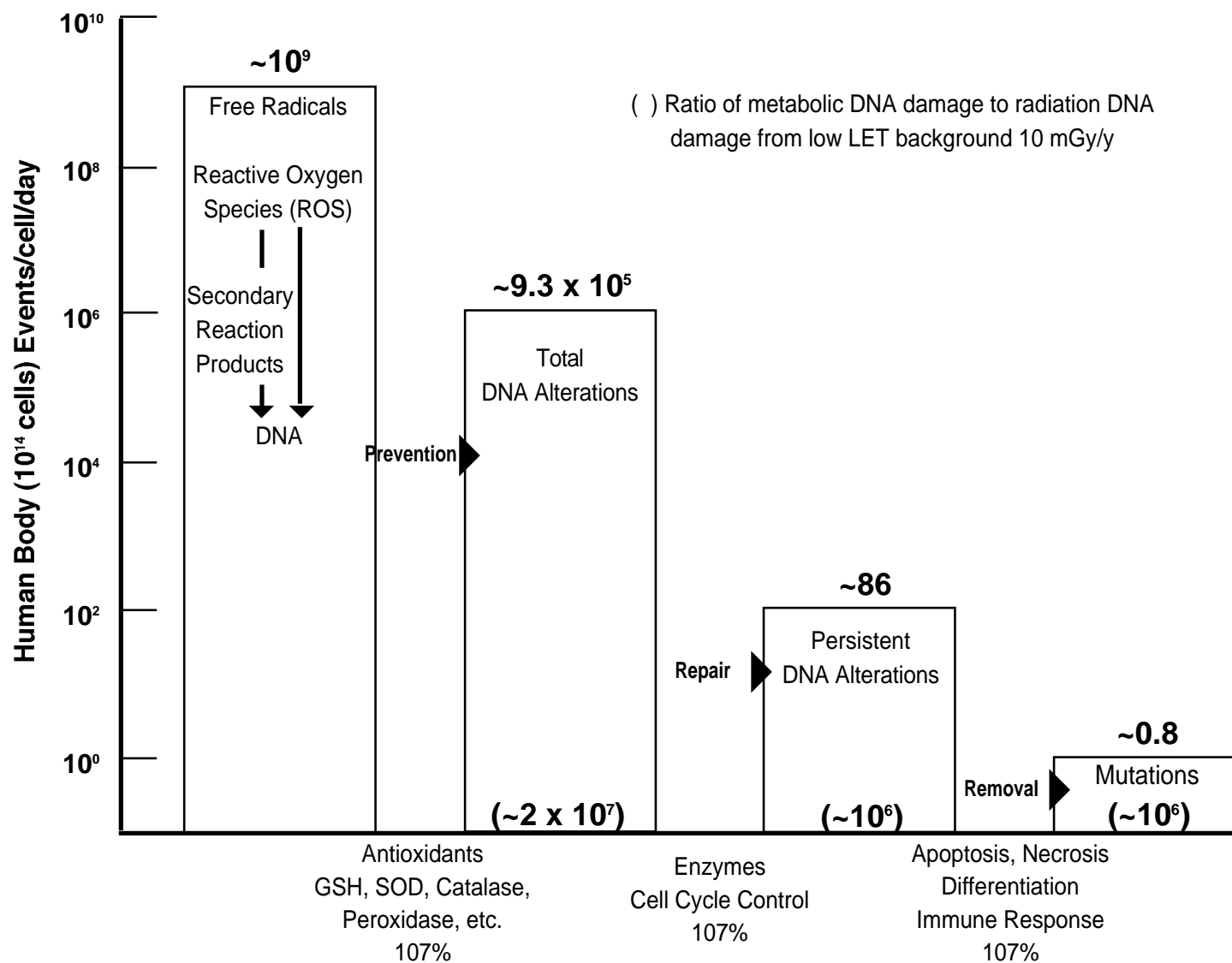


Figure 1. The antimutagenic DNA damage-control biosystem. Estimates based on data in literature.



**Figure 2. The antimutagenic DNA damage-control biosystem response to high background radiation = 120%. Estimates based on data in literature.**

# **RADIATION- INDUCED VERSUS ENDOGENOUS DNA DAMAGE: COMMENTARY ON POLLYCOVE AND FEINENDEGEN**

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Pollycove et al. have provided an excellent comparison of the quantitative data regarding DNA damage due to endogenous sources versus those following low dose ionizing radiation. Their analysis puts into perspective the minute contribution of radiation induced cellular DNA damage at exposures of less than 300 mGy relative to the generation of random damage during normal cellular processes. More importantly, they put DNA damage into a physiological context in which low level ionizing radiation elicits beneficial cellular programs that can detoxify, repair or eliminate damage that offset cumulative damage.

However, while acknowledging the importance of the physiological response, Pollycove et al. imply that the cell is the entity determining biological consequence. As long as a cell is thought to be master of its fate, then the damage, no matter how small, that endangers cell integrity will be thought to be the appropriate basis for modeling risk. I would argue that multicellular organisms integrate and perpetuate damage responses via extracellular signaling mechanisms that are important deterrents to the development of cancer (Barcellos-Hoff and Brooks, 2001). Activation of extracellular signaling has been documented following total body irradiation doses of 100 mGy (Ehrhart, 1997) indicating that signaling via the tissue microenvironment is exquisitely sensitive to small perturbations. Acknowledgment that damage control extends beyond cellular response is critical to modifying the LNT view of radiation risk.

## **REFERENCE**

- Barcellos-Hoff, M. H. and A. L. Brooks. (2001). Extracellular signaling via the microenvironment: A hypothesis relating carcinogenesis, bystander effects and genomic instability. *Radiat. Res.* 156(5):618-627.
- E. J. Ehrhart, A. Carroll, P. Segarini, M. L.-S. Tsang and M. H. Barcellos-Hoff. (1997). Latent transforming growth factor-beta activation *in situ*: Quantitative and functional evidence following low dose irradiation. *FASEB J.*, 11:991-1002 (1997).

# **RADIATION-INDUCED VERSUS ENDOGENOUS DNA DAMAGE: COMMENTARY ON POLLYCOVE AND FEINENDEGEN**

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## **INTRODUCTION**

The article by Pollycove and Feinendegen raises important issues regarding the relative contributions of endogenous DNA damage and radiation-induced DNA to the overall damage burden following low level radiation exposures. Clearly, such considerations and resolution of the issues raised in their paper will have important implications regarding regulatory philosophy. In addition, consideration of the balance between radiation-induced and endogenous DNA damage will impact the design and interpretation of experiments designed to study the effects of low-dose exposures to low LET radiations. Thus, the major contribution of this paper is as vehicle to open debate on significant issues regarding the effects of low level, low LET radiation exposures and the responses of cells to such exposures. However, it would be premature to consider that the conclusions of this paper are final. To their credit the authors agree with this point and state "The emerging model urgently needs rigorous experimental testing, ...". While it is always preferable to specifically design experiments to test a given model, analyzing available data in the context of the proposed model can reveal potential issues for further consideration.

## **RESULTS**

One approach to analyzing available data in the context of the proposed model is to consider dose-limiting studies of DNA damage measured on a cell-by-

cell basis. If one proposes that significant numbers of oxidative DNA lesions are present in cells at a steady state level at any give time, then such damage will be included in the background measure of any DNA damage dependent parameter that is sensitive to these classes of DNA damage. For example, many oxidative DNA lesions will become single-strand breaks in the presence of strong alkali. Thus, assays such as the alkaline comet assay should detect a major fraction of oxidative lesions. In fact, some workers claim to have observed changes in the levels of endogenous DNA damage with this assay (Phillips et al., 1998). In contrast, the formation of micronuclei by DNA damage would reflect double-strand DNA breaks (dsb). Therefore, the background for this assay would include essentially no contribution from endogenous DNA damage due to the low proportion of endogenous lesions (according to the model) that would be dsb. However, the background will include micronuclei that are formed by mechanisms that do not involve DNA damage. The inhibition of the ability of nuclear DNA to rewind supercoils is assayed under neutral conditions (Wright et al., 2000). Thus, only frank single-strand DNA breaks (ssb) will contribute to the parameters measured in this assay. I have chosen these three assays, because they would be expected to have different levels of endogenous DNA damage in their background level due to their sensitivity to different classes of lesions. We can then compare the expected number of lesions per cell, prior to X- or  $\gamma$ -ray exposure, at the dose that gives the minimum statistically significant difference from background, at the dose where the DNA damage dependent parameter is twice background (i.e., the doubling dose) and, when applicable, at the dose that saturates the assay (Table 1). The dose that gives the minimum statistically significant difference above background represents the point at which the radiation-induced DNA damage will make a significant addition to the existing burden of endogenous DNA damage. The doubling dose represents an important consideration for the following reason. If the background value for a given DNA damage-dependent parameter includes the effects (on it) of endogenous DNA damage, then the doubling dose represents the point at which the effect of DNA damage induced by radiation, equals the maximum possible effect of endogenous DNA damage on the parameter being measured. The saturation dose is included because there are a finite number of DNA loops per cell and when all or most of them have at least one ssb then the DNA supercoiling assay will be saturated. Thus, these dose levels represent points in parameter space at which we can determine if the proportions of radiation-induced and endogenous DNA damage, derived from the model of Pollycove and Feinendegen are reasonable or unlikely.

## **DISCUSSION**

The analysis shown in Table 1 suggests that for micronucleus formation and the inhibition of DNA supercoil rewinding the predicted number of DNA

lesions are reasonable (see below). However, examination of the predicted numbers of DNA lesions at the minimum detectable dose and the doubling dose for comet moment and comet length raises some concerns. If the steady state number of oxidations, 24,000, per cell is assumed to be all alkali-labile, and we use the value for radiation-induced DNA lesions of 2 lesions per mGy (Pollycove and Feinendegen, quoted from Ward, 1988), we find that the difference of 12 lesions out of 24,000 would be statistically significant. In other words, the difference between 24,000 and 24,012 would have to be detectable by the comet assay to explain the observed dose-dependent changes in this assay and lesion frequencies used in the model proposed by Pollycove and Feinendegen. Clearly, this is impossible because cell-to-cell variation in the endogenous lesion frequency would be 155 by a Poisson distribution. The 95% confidence interval for such a distribution would be more than 300, i.e., more than 20 times higher than the predicted lesion difference induced by the radiation dose that caused a significant difference in comet moment and comet length. In fact, the lesions predicted to be induced at the doubling dose, for the most part, is also less than the minimum value for the 95% confidence limits of the endogenous damage burden. Thus, the lesion frequencies predicted from the model by Pollycove and Feinendegen are reasonable for the micronucleus assay and the inhibition of DNA supercoil rewinding, appear to be inconsistent with results from the comet assay.

There are three possible explanations for the inconsistency between the comet assay dose response data and the predicted levels of DNA damage predicted by the model of Pollycove and Feinendegen. 1) The alkaline comet assay is sensitive to only a small fraction of the endogenous lesions. 2) The frequency of DNA lesion induction by ionizing radiation is too low. 3) The steady-state number of endogenous lesions is too high. In terms of the first possibility, work (Sardas et al., 2001) shows that the comet assay can detect increases in oxidative DNA damage in diabetic patients and its reduction by vitamin E. Further work with this assay can demonstrate the protective effects of dietary antioxidants on oxidative DNA damage (Szeto et al., 2002). Thus, it seems unlikely that the comet assay would not be sensitive to at least some endogenous oxidative DNA damage. While the possibility that a significant fraction of the endogenous lesions could be masked from the comet assay by nuclear matrix-DNA anchoring proteins cannot be ruled out per se, it seems unlikely. First, the fraction of unmasked lesions would have to be on the order of 0.001. Second, when the assay is run with or without proteinase K or longer and shorter lysis time is used, the relative changes in the response to radiation-induced DNA are inconsistent with such a large masking factor.

In consideration of the levels of radiation-induced DNA damage (possibility 2), it is important to note that the values that Pollycove and Feinendegen used for radiation-induced values was quoted from Ward (1988). This value appears to be low, because Ward only consid-

ered an incomplete list of possible radiation-induced base damages. While one can argue that a complete list has yet to be delineated, Ward only considered 2 types of base damage: dihydrothymine and 8-hydroxyadenine. Further, the values used by Ward for dihydrothymine formed per Gy are 4-fold lower than other estimates based on work from the same laboratory as that quoted by Ward (Roti Roti and Cerutti, 1974). Considering that there could be easily 10-fold more types of base damages and that bases are 3- to 8-fold more likely to site of attack for the formation of ssb by (OH radicals (Michalik et al., 1995), a much larger base damage to ssb ratio should be used for radiation-induced DNA damage. Also, from the point of view of the comet assay per se, it seems unlikely that 12 DNA damages per cell would be sufficient to significantly enhance DNA migration. Thus a 6-10-fold larger (or more) estimate of DNA lesions per mGy than that quoted by Pollycove and Feinendegen is in order. It is also worth noting that the estimate of DNA damage per mGy are based on extrapolations from damage measurements after relatively high radiation doses, assuming that 0 Gy = 0 lesions. If these extrapolations were made to a given level of endogenous DNA damage they would result in larger estimates of DNA damage per mGy.

In consideration of the levels of endogenous DNA damage (possibility 3), the value of 24,000 DNA damages per cell used by Pollycove and Feinendegen was derived from a large body of work. However, it appears that all of this work utilized assays for DNA lesions on bulk, isolated DNA rather than cell-by-cell assays. One potential problem with bulk assays for DNA damage is the possibility that heavy damage in a small number of cells will increase the estimate of the average damage per cell. For example, consider 24,000 lesions per cell obtained from measuring lesions in  $10^7$  cells, or  $2.4 \times 10^{11}$  lesions. If 2% of the cells, from which the DNA was isolated were dying, (i.e.,  $2 \times 10^5$  cells) and there were  $10^6$  DNA lesions per dying cell (i.e., 1 damaged base per thousand), then the dead cells would contribute  $2 \times 10^{11}$  lesions to the assay. Then there would be  $4 \times 10^{10}$  lesions per  $0.98 \times 10^7$  viable cells, or 4,080 lesions per cell. Thus a 1-2 % fraction of dead or dying cells could cause 2- to 6-fold over estimate of endogenous DNA damage per cell. Based on a reading of the methods sections of the papers quoted by Pollycove and Feinendegen to estimate the level of endogenous DNA damage per cell (Atamna et al., 2000; Beckman and Ames, 1997; Helbock et al., 1998; Jaruga and Dizdaroglu, 1996; Kasprzak et al., 1992; Olinski et al., 1995; Wilson and Barsky, 2001), it was not obvious that careful measurements of the dead cell fraction was made in any of these studies. Further, some of the in vivo studies used methods of euthanasia that could induce DNA in tissues such as the brain. Thus it seems possible that the value of 24,000 endogenous DNA lesions per cell under steady-state conditions is high.

## SUMMARY AND CONCLUSION

Given that the present writing is a comment on the

paper by Pollycove and Feinendegen rather than a fully developed alternative model, further details on the points raised and discussion of counter arguments are best left for future work. The goal in introducing these issues is not to be negative to the paper but to present a basis for future discussions and more importantly future experimental work. In fact, the basic conclusion of the Pollycove and Feinendegen paper that the effects of radiation-induced DNA and the cellular responses to such DNA damage needs to be considered in the context of a steady-state base line of endogenous DNA damage is clearly supported. Such considerations would potentially impact regulatory philosophy. The questions raised here relate to the relative ratio of radiation-induced to endogenous DNA damage. While many good arguments can be made to push this ratio higher or lower, such debate can only be resolved by definitive experimental results.

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## REFERENCES

1. Atamna H, Cheung I and Ames BN: A method for detecting abasic sites in living cells: Age-dependent changes in base excision repair. *Proceedings of the National Academy of Science* 2000; 97:686-691.
2. Beckman KB and Ames BN: Oxidative decay of DNA. *Journal of Biological Chemistry* 1997; 272:19633-19636.
3. Bisht KS, Moros EG, Straube WL and Roti Roti JL: The effect of 835.62 MHz FDMA or 847.74 MHz CDMA modulated radiofrequency radiation on the induction of micronuclei in C3H 10T-1/2 cells. *Radiation Research* 2002; 157:506-515.
4. Helbock HJ, Beckman KB, Shigenaga MK, Walter PB, Woodall AA, Yeo HC and Ames BN: DNA oxidation matters: The HPLC-electrochemical detection assay of 8-oxo-deoxyguanosine and 8-oxo-guanine. *Proceedings of the National Academy of Science USA* 1998; 95:288-293.
5. Jaruga P and Dizdaroglu M: Repair of products of oxidative DNA base damage in human cells. *Nucleic Acids Research* 1996; 24:1389-1394.
6. Kasprzak KS, Diwan BA, Rice JM, Misra M, Riggs CW, Olinski R and Dizdaroglu M: Nickel(II)-mediated oxidative DNA base damage in renal and hepatic chromatin of pregnant rats and their fetuses. Possible relevance to carcinogenesis. *Chemical Research Toxicology* 1992; 5:809-815.
7. Li L, Bisht KS, Zhang P, Straube WL, Moros EG and Roti Roti JL: Measurement of DNA damage in mammalian cells exposed to radiofrequency fields at high SAR in vitro. *Radiation Research* 2001; 156:328-332.
8. Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF and Roti Roti JL: Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation. *Radiation Research* 1997; 148:608-617.
9. Malyapa RS, Bi C, Ahern EW and Roti Roti JL: Detection of DNA damage by the alkaline comet assay after exposure to low-dose gamma radiation. *Radiation Research* 1998; 149:396-400.
10. Michalik V, Tartier L, Spothem-Maurizot M, and Charlier M: Reaction of hydroxyl radical with B- and Z-DNA. In: *Radiation Damage in DNA: Structure/Function Relationships at Early Times*, eds., Fuciarelli AF and Zimbrick JD, Battelle Press, 1995.
11. Olinski R, Zastawny TH, Foksinski M, Barecki A and Dizdaroglu M: DNA base modifications and antioxidant enzyme activities in human benign prostatic hyperplasia. *Free Radical Biology Medicine* 1995; 18:807-813.
12. Plappert U, Raddatz K, Roth S and Fliedner TM: DNA-damage detection in man after radiation exposure - the comet assay - its possible application for human biomonitoring. *Stem Cells* 1995; 13:215-222.
13. Pollycove M and Feinendegen LE: Radiation-induced versus endogenous DNA damage: Possible effect of inducible protective responses in mitigating endogenous damage. Submitted.
14. Phillips JL, Ivaschuk O, Ishida-Jones T, Jones RA, Campbell-Beachler M and Haggren W: DNA damage in Molt-4 T-lymphoblastoid cells exposed to cellular telephone radiofrequency fields in vitro. *Bioelectrochemistry and Bioenergetics* 1998; 45:103-110.
15. Roti Roti JL and Cerutti PA: Gamma-ray induced thymine damage in mammalian cells. *International Journal of Radiation Biology* 1974; 25:413-417.
16. Roti Roti JL and Wright WD: Visualization of DNA loops in nucleoids from HeLa cells: Assays for DNA damage and repair. *Cytometry* 1987; 8:461-467.

**Table 1: Predicted Endogenous and Radiation-Induced DNA Damage Levels at Doses that Induce Specific Changes in DNA Damage Dependent Parameters**

Assay / Parameter	Background	Minimum Dose	Doubling Dose	Maximum Dose
Exponentially Growing C3H 10T1/2 Cells				
Alkaline comet: Moment <sup>^</sup> DNA alterations/cell*	1.15 $\mu$ m 24,000	1.75 $\mu$ m @ 0.6 cGy 24,012	2.30 $\mu$ m @ 1.8 cGy 24,036	n/a
Alkaline comet: Length+ DNA alterations/cell*	26 $\mu$ m 24,000	31.8 $\mu$ m @ 0.6 cGy 24,012	41.0 $\mu$ m @ 30 cGy 24,600	n/a
Rat Lymphocytes				
Alkaline comet: Moment+ DNA alterations/cell*	0.42 $\mu$ m 24,000	0.6 $\mu$ m @ 0.6 cGy 24,012	0.84 $\mu$ m @ 1.0 cGy 24,020	n/a
Alkaline comet: Length+ DNA alterations/cell*	20.5 $\mu$ m 24,000	29.0 $\mu$ m @ 0.6 cGy 24,012	41.0 $\mu$ m @ 5.5 cGy 24,110	n/a
Exponentially Growing C3H 10T1/2 Cells				
Micronuclei: %cells w MN** Dsb/cell*	2.5 0.1	4 @ 30 cGy 12	5 @ 40 cGy 16	n/a
Plateau Phase C3H 10T1/2 Cells				
Micronuclei: %cells w MN** Dsb/cell*	4 0.1	10 @ 90cGy 38	8 @ 70cGy 28	n/a
Exponentially Growing HeLa Cells				
DNA supercoil rewinding <sup>^^</sup> Ssb/cell*	500	100 cGy 1,500	500 cGy 5,500	2000 cGy 20,500
DNA loops w ssb/10,000	0.5	0.14	0.42	0.87

\*Calculated from Pollycove and Feinendegen, current Ms

<sup>^</sup>Malyapa et al., 1997; see also Li et al., 2001

+Malyapa et al., 1998; see also Singh et al., 1994; Plappert et al., 1995

\*\*Bisht et al., 2002

<sup>^^</sup>Roti Roti and Wright, 1987

# RADIATION-INDUCED VERSUS ENDOGENOUS DNA DAMAGE: COMMENTARY ON POLLYCOVE AND FEINENDEGEN

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Pollycove and Feinendegen estimated the amount of DNA damages in living cells produced by ionizing radiation at the environmental background level and showed how small it is as compared with that from naturally occurring events, mainly from reactive oxygen species (ROS). The calculations, using available experimental data and assumptions, are rigorous even with micro-dosimetry considerations. Their analysis yields a highly interesting figure for the health effect of ionizing radiation in the natural environment. In consequence, the authors claimed that a low dose of ionizing radiation at the environmental level is harmless. This point has been repeatedly discussed by other authors (e.g. Billen<sup>1</sup>, Kondo<sup>2</sup>) and at scientific meetings specially focused on this subject. I find myself in complete agreement with the authors' idea and as the authors point out, it is an idea that has never been incorporated in the radiation protection policy. The presentation of this article is quite timely and the authors' efforts are highly regarded at the current juncture of revising the radiation protection policy by incorporating new biological findings.

In this article, the authors made a new approach to estimating the amount of initial DNA damages and its final amount after modification by repair. At the beginning, they showed the amounts of endogenous oxidative DNA adducts in the steady state (Table 1). To estimate the initial amount of these damages from the measured

amount of the steady state damages, the authors applied the factor of repair,  $10^4$ , which was estimated from the half life of the damages, 25 min.<sup>3</sup> They gave the amount of endogenous oxidative adducts from two different calculations, i. e.,  $OX/cell/d = 10^6$ .

Here, I would like to mention oxidative damages to precursor nucleotides in DNA synthesis, such as 8-OH dGTP, which occupy an important part of oxidative damage. These damages would create a very high frequency of spontaneous mutations, i. e., 100-10000 fold higher, if they are not removed by the cellular function of the *mutT* gene<sup>4</sup> in *E.coli* or its homolog in humans.<sup>5</sup> Although this consideration will not change the final value of the E/R ratio given by the authors in Table 5, the amount of the total initial damage becomes much larger if the DNA precursor damage is taken into account. The contribution of the oxidative DNA precursor damages to the final mutation/cancer risk will become clear by experiments with animals lacking this repair function.

On the other hand, DNA adducts produced by background radiation cannot be measured, because their formation is hindered by those produced by endogenous ROS. The amount of radiation-induced initial DNA damages was assumed to be 2 DNA alterations/cell/mGy before repair. The authors applied a factor 500 for repair. Here, measured values for radiation-induced DNA base alterations are available from acute low LET irradiation experiments. The most reliable experimental value for thymine glycols obtained with the sensitive detection method is  $6.4 \times 10^{-1}$  TG/mGy/cell.<sup>6</sup> Half life values are available for thymine glycols in cultured human cells, 60 min<sup>7</sup> and for 8-oxoguanines in the mouse liver, 20 min.<sup>8</sup> These half lives are, as the authors quoted (Table 1), of the same order of magnitude as that for ROS-induced damages, i. e., 25 min, and can be applied to the estimation of the DNA base damage in the steady state, i. e., continuous irradiation at 1 mGy/year, applying the same calculation as used for estimation of the amount of the total ROS-induced DNA damage from the steady state damages. This estimation gives a two order of magnitude lower value for background radiation damages than that of the authors' estimation (Table 5) and, therefore, a higher value of E/R ratio for total steady state DNA alterations. It should be noted that the repair activity can be enhanced by a low dose of radiation.<sup>6</sup>

More important is DSB of DNA. The authors used the yield of single strand breaks (SSB) and their repair half life (5 min) for calculation of the daily formation of SSB at the steady state, and estimated from this value the initial daily formation of DSB per cell, i. e.,  $DSB = 0.1$ . A repair factor of 10 was further applied. The same repair factor was also used for radiation-induced DSB. This factor seems to be reasonable from the slow repair of DSB (half life: 2 h).<sup>9</sup> The authors showed that the portion of low LET background radiation-induced DSB among total ROS-mediated DSB is still  $10^3$  fold smaller. Recently, the genetic control of the DSB-rejoining

process has become clear with regard to cellular recovery from lethal damage, although it may not fall within the scope of this paper. It should be noted that DSB is frequently produced as an intermediate during DNA replication and its rejoining occurs with high fidelity. This replication-associated DSB frequency during the cell cycle is thought to be considerably high, compared with that of endogenous ROS-mediated DSB. The rejoining system for this replicative DSB is thought to function for repair of ROS- and radiation-induced DSB.

As the end point of DNA damage, mutations from the endogenous origin and background radiation are considered in relation to cancer (Figs. 1 and 2). As the authors pointed out, cells with persistent DNA alterations are removed by apoptosis, and cells with cancer mutation are subjected to immuno-suppression. Therefore, the final risk of mutation and, consequently, cancer is reduced down to a level farther than that directly expected from the amount of initial DNA damage. Recently, the lower mutation frequency in embryonic stem cells compared with that of somatic cells was demonstrated.<sup>10</sup> Therefore, the cancer-associated mutation in stem cells is expected to occur with much lower frequency than that shown in Figures 1 and 2.

Finally, the authors stated that the linear no-threshold (LNT) hypothesis is invalid for such complex adaptive systems as the mammalian organism. Since the initial amount of DNA damage is thought to be linearly proportional to the radiation dose, it is questioned whether or not the biological end point, such as mutation and cancer, is suppressed specifically at low doses in the manner of adaptive response, including activation of DNA repair, apoptosis, and immunological activities. Experimental data are now needed for the dose-response of these activities at various dose-rates.

The shape of the dose-response curve for cancer induction at the low dose region is now an important issue, because it will determine the cancer risk of low dose radiation with special regard to the presence of a threshold dose. The threshold problem was approached from the experimental evidence of the threshold dose-response of malformation in fetal mice.<sup>2,11</sup> In my review of cancer data<sup>12</sup>, an approach was made from non-tumor doses, defined as the highest dose at which no statistically significant tumor increase is observed, which increased with lowering a dose-rate by a factor of 10-100 with whole-body low-LET radiation. This dose-rate effect appears to exist also for high LET radiation at a very low dose-rate. The presence of the threshold dose seems now to be certain.

The authors' efforts to quantify DNA damages in the human environment led us to understand the reality of radiation risk and are expected to extend further to the clarification of the whole scheme of cancer risk. This paper is a milestone of scientists' efforts to understand nature.

## REFERENCES

1. Billen D, Spontaneous DNA damage and its significance for the "negligible dose". Controversy in radiation protection. *Radiat. Res.* 1990; 124: 242-245.
2. Kondo S, Health Effects of Low-level Radiation, 1993, Kinki University Press, Osaka, and Medical Physics Publishing, Madison, WI.
3. Beckman KB, Ames BN. Oxidative decay of DNA, *J. Biol. Chem.* 1997; 272: 19633-19636.
4. Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature* 1992; 355: 273-275.
5. Mo JY, Maki H, Sekiguchi M. Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool. *Proc. Natl. Acad. Sci. USA*, 1992; 89: 11021-11025.
6. Le XC, Xing JZ, Lee J, Leadon SA, Weinfeld M, Inducible repair of thymine glycol detected by an ultrasensitive assay for DNA damage. *Science* 1998; 280: 1066-1069.
7. Leadon SA, Hanawalt PC, Monoclonal antibody to DNA containing thymine glycol, *Mutation Res.* 1983; 112: 191-200.
8. Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H, Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. *Carcinogenesis* 1986; 7: 1849-1851.
9. Blocher D, Pohl W. DNA double strand breaks in Ehrlich ascites tumour cells at low doses of X-rays, II. Can cell death be attributed to double strand breaks. *Int. J. Rad. Biol.* 1982; 42: 329-338.
10. Cervantes RB, Stringer JR, Shao C, Tischfield JA, Stambrook PJ, Embryonic stem cells and somatic cells differ in mutation frequency and type, *Proc. Natl. Acad. Sci. USA* 2002; 99: 3586-3590.
11. Kondo S. Evidence that there are threshold effects in risk of radiation. *J. Nucl. Sci. Technol.* 1999; 36: 1-9.
12. Tanooka H, Threshold dose-response in radiation carcinogenesis: An approach from chronic - irradiation experiments and a review of non-tumor doses. *Int. J. Rad. Biol.* 2001; 77: 541-551.

# **RADIATION- INDUCED VERSUS ENDOGENOUS DNA DAMAGE: COMMENTARY ON POLLYCOVE AND FEINENDEGEN**

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The premises underlying the article by Pollycove and Feinendegen are sound, scientifically current, the basis for considerable research both in the past and currently, and are unlikely to generate disagreement among many or any scientists.

\* It is difficult, if not impossible, to accurately and absolutely determine all the biological effects of low doses of radiation to isolated cells or human populations.

\* "Biology" itself generates damage to biological systems.

\* Biological systems have a remarkable and complex capacity for responding to damage that goes well beyond DNA repair.

\* The linear-no-threshold model for estimating the

effects (and risks) of low doses of radiation is based on conservative assumptions about the effects of radiation at high and low doses and has not been conclusively proven.

However, these authors do little to advance the current state of knowledge, or lack thereof, on these premises, and in the end can only conclude that additional experimentation is needed to resolve the scientific uncertainties that they represent.

This is certainly an important topic. Resolution of the low dose radiation dilemma is an ongoing scientific challenge and, more to the point, an important public policy issue since it is at the heart of our efforts to adequately and appropriately protect people and the environment from the effects of low doses of radiation while making the best use of available resources.

The authors do a good job of reviewing a considerable body of scientific literature and using available data in order to "do the math" of radiation-induced and endogenous DNA damage, providing numerical estimates and comparisons of DNA damages, DNA breaks, and DNA repair. But detailed calculations can only take you so far and are only as good as the available biology which we contend is not yet up to close numerical scrutiny. Several truisms will serve to make the point that biology is indeed difficult.

\* Understanding the chemistry of radiation's interaction with biological systems is not the same as having a detailed mechanistic understanding of the underlying biology.

\* Sequencing of the human genome and other whole genomes has further emphasized the complexity of gene network interactions - feedback loops, redundant or alternate pathways, etc. Layers of regulation are seen at various levels: intra-cellular (gene transcription via promoters, enhancers, etc., gene translation, post-translational protein modification, protein complexes), inter-cellular/tissues (extracellular matrix, cell-cell communication, etc.), organs and whole organisms (autocrine, paracrine signaling).

\* The real issue for radiation-induced (and likely also endogenous) damage may be more one of quality than of quantity, i.e., rare, perhaps even undetected or generally uncharacterized lesions may be the risk factors for low doses of radiation rather than the commonly measured and characterized lesions.

Herein lies the challenge for Pollycove and Feinendegen and the difficulty. "Doing the math" of radiation-induced and endogenous damage is an informative and enlightening process and one that can certainly generate testable experimental hypotheses. However, "the numbers" should in no way be confused with proof or disproof for or substituted for experimental demonstrations of biological mechanisms.

Similarly, and this criticism can be directed at most of us who have conducted or supported radiation biology experiments over the years, in vitro mechanisms are not to be confused with in vivo mechanisms. Many of the studies referenced by Pollycove and Feinendegen are in

vitro studies and can only take us so far in truly understanding the biological effects and mechanisms of low doses radiation exposure. Just as our understanding of high dose radiation exposures provides us with an admittedly limited understanding of the critical events that occur at low dose of radiation, so too in vitro experiments only provide a limited view of the critical events that occur in vivo following exposure to low doses of radiation.

Pollycove and Feinendegen raise a number of current and important questions for low dose radiation biology.

\* Do we know the full range of lesions induced by low doses of radiation? by endogenous processes? Do we know the efficiency and accuracy of repair of all of these lesions? Do we know the biological effects of all of these lesions?

\* Why doesn't damage induced by normal endogenous processes constitutively induce adaptive type responses in cells like those induced by low doses of ionizing radiation? If there is something unique about radiation-induced damage that is responsible for this phenomenon, then is it even reasonable to compare the "numbers" between radiation-induced and endogenous damage?

\* How can we explain low dose bystander effects or gene inductions that are induced by radiation if those same biological systems are already "overwhelmed" or primed with similar, but greater numbers of damages being induced by endogenous factors? Again, this suggests that there are significant qualitative differences between radiation-induced and endogenous damage making numerical comparisons irrelevant.

Just as we need new experimental strategies and results to help us understand the biological effects and mechanisms of low doses of ionizing radiation, so too do we need quantitative analyses of complex biological processes and metabolic phenomena. At the same time, we should not confuse either of these important research approaches as a substitute for the other. It is not apparent that this distinction is maintained in the manuscript by Pollycove and Feinendegen.

# COMMENTARY AND RESPONSE TO REVIEWER CRITIQUES RE "RADIATION- INDUCED VERSUS ENDOGENOUS DNA DAMAGE: POSSIBLE EFFECTS OF INDUCIBLE PROTECTIVE RESPONSES IN MITIGATING ENDOGENOUS DAMAGE"

by M. Pollycove and L. E. Feinendegen

We are pleased to have the opportunity to respond to the comments from Drs. M. H. Barcellos-Hoff, J.L. Roti Roti, H. Tanooka, and D. G. Thomassen and N.F. Metting.

Regarding the comments of Dr. M.H. Barcellos-Hoff, we fully concur with the need to emphasize the important role of intercellular and matrix signaling. We attempted to acknowledge the importance of signaling as we knew it at the time of writing our paper. For instance, we referred in our paper to intercellular and tissue signaling repeatedly, for instance, in the "Introduction" and sections "Quantitative aspect of DNA damage; Ionizing radiation", "DNA damage control system"; "The effect of low-dose of low-LET radiation on the damage control system"; "Discussion"; and "Summary". We unfortunately did not know the excellent paper by Barcellos-Hoff and Brooks at the time of our writing the paper (Barcellos-Hoff and Brooks, 2001); we surely would have referred to it and are glad to do it now. We consider the summarizing review by Barcellos-Hoff and Brooks a concise account on tissue being a complex system with cellular and

non-cellular elements with different radiation sensitivities and responses, but reacting as a whole. We used in our paper terms such as "indirect effects" and "intercellularly operating factors"; they include all tissue-related signaling effects. We share the argument by these authors that the stochastic effects of ionizing radiation in tissues produce cell effects that derive from all tissue factors induced by ionizing radiation, in contrast to cellular effects seen in studies with dedicated microbeam radiation that are directed to single cells and single cell responses including bystander effects.

The comments of Dr. Roti Roti furnish new avenues for analyzing various types of DNA damage by low-dose irradiation. We are pleased with the concordance between our data estimates on endogenously induced double strand breaks, DSBs, with the data on micronucleus formation that reflects the presence of DSB. We acknowledge the disagreement between our estimates and the data based on the ingenious application of the alkaline comet assay data. We appreciate Dr. Roti Roti's efforts to explain this discrepancy. We believe this is very useful for further experimental work. In taking the liberty of expanding Dr. Roti Roti's first of his three possible explanations of the inconsistency, we express our belief that it is conceivable that existing DNA oxyadducts at the steady state level may be sensitive to abrupt changes in intracellular homeostatic equilibrium brought about by handling cells in preparing them for the alkaline comet assay. For instance, minute alterations of temperature, pH, substrate and/or ionic composition of the culture medium into which cells are harvested after *in vivo* treatment, can result in an immediate drastic biochemical response. This was expressed, for instance, by the immediate elimination of the low-dose induced delayed and temporary depression of thymidine kinase activity in mouse bone marrow cells, when they were isolated into suboptimal culture media at various times after low-dose and low LET irradiation *in vivo* (Feinendegen et al. 1984). In addition to our possible underestimate of the number of radiation-induced base changes, for which we used earlier data from Ward (Ward 1988), Dr. Roti Roti pointed to the possible neglect of the contribution of dead cell DNA in the assay of endogenously generated DNA alterations. It is indeed conceivable that altered DNA from dead cells in the material to be assayed could lead to a large overestimation, perhaps by an order of magnitude, of endogenously generated DNA alterations. But according to our analysis, this would still not materially change the enormous preponderance regarding both quantity and to a lesser extent quality of endogenous DNA alterations compared to those induced by background radiation exposure. According to Dr. Roti Roti's analysis, our predicted 1000:1 ratio of endogenous to background DSB remains reasonable.

We also welcome Dr. Tanooka's positive assessment of our estimates. His reference to oxidative damages to precursor nucleotides for DNA synthesis provides an additional source for endogenous DNA damage. As he

points out, such precursors may be imperfectly repaired and could significantly increase the number of DNA alterations. His estimate of background radiation-induced DNA alterations that are based on radiation-induced thymine glycol formation and some of our assumptions give a lower value than our estimate on radiation-induced base changes. This supports the notion that endogenous alterations far outweigh those of background radiation. Furthermore, Dr. Tanooka indicates that in addition to endogenous ROS being responsible for non-radiogenic DSB, considerable production of DSB arises during DNA replication in the cell cycle with imperfect repair potential, thus supporting our estimated ratio of DSB's derived from endogenous ROS and irradiation. The very recent report on embryonic stem cells having a lower frequency of mutations than somatic cells would likely be applicable to tumorigenic stem cells, as Dr. Tanooka indicated. This would suggest that our estimate of about one mutation per cell per day from endogenous sources would be too high for stem cells that may acquire oncogenic transformation. However, Dr. Tanooka's higher estimate for the production of DNA alterations from endogenous sources may well balance the reduced incidence of mutation in stem cells. A final comment pertains to Dr. Tanooka's reference to adaptive responses. In our opinion the term adaptive response should include damage prevention by cellular defenses, in addition to enzymatic DNA damage repair and damage removal by apoptosis and immune surveillance. We too ascribe these protective mechanisms to operate at both single and protracted exposures and are largely responsible for the threshold dose response reported by Dr. Tanooka in his recent review on tumor induction by chronic beta irradiation (Tanooka 2001).

The commentary of Drs. Thomassen and Metting principally agrees with our assessments being in line with current research. We are glad that these authors went further and directed attention to important future work that needs to be done, as we also state in our paper as a consequence of the estimates on DNA damage from different sources. The crucial question in the context of our paper directs attention to protective responses to affect endogenously induced DNA damage rather than that caused by low doses of ionizing radiation. We can only agree with the need for linking phenomena to biologically explainable mechanisms. The enormous complexity of biological tissue as an adaptive system characterized by most intricate signaling networks at all levels of biological organization seems insurmountable; to understand the workings of this system will surely be much promoted by the new radiobiological research that is currently under way especially with the help of the US Department of Energy (DOE). We can only agree that this research continues to require much additional investment in new models and techniques and the need to increasingly investigate complex tissue systems rather than cells in culture. Our paper attempts to draw attention to the need for analyzing responses to low doses in

complex tissues. From what we know at present we should view reactions of such systems in linear as well as non-linear terms at such low dose levels where the signaling networks are modulated rather than destroyed, as commonly seen at high doses. In this context, we also like to emphasize that protective or adaptive responses that appear at low but not at high doses need to be seen more as a consequence of signal changes in cells and tissues rather than of damage, the incidence and severity of which, for instance, in DNA is extremely small at low doses compared with the damage from non-radiogenic sources. As indicated in our paper, the delayed appearing and temporary lasting protective responses specific for low doses are considered to belong to the physiologic stress response system. The fluctuations of endogenous ROS production in normal mammalian cells such as through ROS minibursts challenge homeostasis and elicit a broad spectrum of cellular responses that are likely ubiquitous and not primarily initiated by low levels of DNA damage (Finkel and Holbrook 2000). The low-dose-induced changes in cellular signaling appear to fall into this category of responses to suprabasal ROS bursts as they also occur in amounts of some 70 to 200 ROS per low-LET particle tracks of  $\mu\text{m}$  ranges in vivo stochastically throughout cells and matrix, each burst within much less than a microsecond. By comparison, oxidative metabolism alone creates endogenous ROS in extramitochondrial cytoplasm an average rate of about 10 per second throughout the cell (Feinendegen, 2002). We are confident that the new DOE research program on the biological effects of low-dose radiation will have far reaching consequences beyond the field of radiation biology.

## REFERENCES

- Barcellos-Hoff, M.H., Brooks, A.L. (2001). Extracellular signaling through the microenvironment: A hypothesis relating carcinogenesis; bystander effects, and genomic instability. *Radiat. Res.*, 156, 618-627.
- Feinendegen, L.E., Muehlensiepen, H., Lindberg, C., Marx, J., Porschen, W., Booz, J. (1984). Acute and temporary inhibition of thymidine kinase in mouse bone marrow cells after low-dose exposure. *Int. J. Radiat. Biol.*, 45, 205-215.
- Feinendegen, L.E. (2002). Reactive oxygen species in cell responses to toxic agents. *Human & Exper. Toxicol.* 21, 85-90.
- Finkel, T., Holbrook, N.J. (2000). Oxidants, oxidative stress and the biology of aging. *Nature (London)*, 408, 239-247.
- Tanooka, H. (2001). Threshold does-response in

radiation carcinogenesis: An approach from chronic  $\text{I}^{125}$ -irradiation experiments and a review of non-tumor doses. *Int. J. Rad. Biol.*, 77, 541-551.

- Ward, J.,F. (1988). DNA damage produced by ionizing radiation in mammalian cells: Identities, mechanisms of formation, and repeatability. *Prog. Nucl. Acid Res. and Mol. Biol.*, 35, 95-125.

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# NON-LINEAR DOSE-RESPONSE RELATIONSHIPS IN BIOLOGY, TOXICOLOGY AND MEDICINE

*An International Conference*

May 28-30, 2003

Murray D. Lincoln Campus Center, University of Massachusetts, Amherst, MA

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**Lunch: \$16 each day** ☐ Wednesday ☐ Thursday **Lunch Total** \_\_\_\_\_

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For more conference information contact: BELLE Office

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