

3-2008

NON-PROBLEMATIC RISKS FROM LOW-DOSE RADIATION-INDUCED DNA DAMAGE CLUSTERS

Daniel P Hayes

New York City Department of Health & Mental Hygiene, New York, NY

Follow this and additional works at: https://scholarworks.umass.edu/dose_response

Recommended Citation

Hayes, Daniel P (2008) "NON-PROBLEMATIC RISKS FROM LOW-DOSE RADIATION-INDUCED DNA DAMAGE CLUSTERS," *Dose-Response: An International Journal*: Vol. 6 : Iss. 1 , Article 4.
Available at: https://scholarworks.umass.edu/dose_response/vol6/iss1/4

This Article is brought to you for free and open access by ScholarWorks@UMass Amherst. It has been accepted for inclusion in Dose-Response: An International Journal by an authorized editor of ScholarWorks@UMass Amherst. For more information, please contact scholarworks@library.umass.edu.

Dose-Response, 6:30–52, 2008
Formerly *Nonlinearity in Biology, Toxicology, and Medicine*
Copyright © 2008 University of Massachusetts
ISSN: 1559-3258
DOI: 10.2203/dose-response.07-023.Hayes

International **Dose-Response** Society
www.dose-response.org

NON-PROBLEMATIC RISKS FROM LOW-DOSE RADIATION-INDUCED DNA DAMAGE CLUSTERS

Daniel P. Hayes □ Office of Radiological Health, New York City Department of Health & Mental Hygiene, New York, NY

□ Radiation-induced DNA damage clusters have been proposed and are usually considered to pose the threat of serious biological damage. This has been attributed to DNA repair debilitation or cessation arising from the complexity of cluster damage. It will be shown here, contrary to both previous suggestions and perceived wisdom, that radiation induced damage clusters contribute to non-problematic risks in the low-dose, low-LET regime. The very complexity of cluster damage which inhibits and/or compromises DNA repair will ultimately be responsible for the elimination and/or diminution of precancerous and cancerous cells.

Keywords: radiation-induced damage clusters, radiation risks, DNA repair, low-dose radiation, low-LET radiation, hormesis

INTRODUCTION

Complex DNA damage clusters arise from the absorption of ionizing radiation in biological organisms. It has been suggested that these clustered damages are less repairable and hence more likely to lead to biological consequences (Goodhead and Nikjoo, 1997; United Nations, 2000). As will be developed here and contrary to previous suggestions, there are reasons to believe that low-LET radiation-induced clusters pose non-problematic risks at low radiation doses and may even be a *sine qua non* for reducing/eliminating risks at these radiation levels.

The topic being considered here assumes great current importance in the field of radiation protection. Presently there are two camps with diametrically opposing views on risk from low-dose radiation. One holds the view that there may be significant risk; the other holds that there is little, if any, evidence of risk at these radiation levels. The former of these two camps is exemplified by the recent *BEIR VII REPORT* of the American National Academy of Sciences (NRC, 2006); with the latter exemplified by the recent report of the French Academy of Sciences and the French Academy of Medicine (Tubiana et al., 2005), and that of Feinendegen (2005). The results presented here are relevant to the resolution of these opposing viewpoints, in actual fact lending credence to non-problematic risk from low-dose radiation.

Address correspondence to Daniel P. Hayes, Office of Radiological Health, New York City Department of Health & Mental Hygiene, 2 Lafayette St., New York, NY 10007. Phone: 212 676-1558; fax: 212 676-1548; e-mail: dhayes@health.nyc.gov.

Nonproblematic risks from radiation damage clusters

Successive sections of this paper will be devoted to the following topics: the definition and quantification of low-dose radiation, the definitional basis of radiation-induced clusters arising from biophysical modeling, the conceptual basis of laboratory studies of radiation-induced clusters, repair in relation to radiation-induced clusters, laboratory observations pertinent to radiation-induced clusters, and discussion and conclusions.

LOW-DOSE RADIATION

Radiation doses of the order of several millisieverts (mSv) will, at most, produce very minor increases in the frequency of untoward health effects and are probably too small to be estimated directly from epidemiological data, being buried in the noise of the background risk. A dose of 100 mSv is some 100 times the average annual worldwide low-LET (Linear Energy Transfer) background (0.9 mGy). (For low-LET radiation, X-rays and γ -rays, effective doses in Sieverts (Sv) are taken to be equal to absorbed doses in Grays (Gy).) The maximal permissible radiation levels recommended in the United States by the National Council on Radiation Protections and Measurements (NCRP) for exposure to radiation other than background radiation and from medical applications are 1 mSv per year for the general population and 50 mSv per year for radiation workers (Federal Register, 1987). The International Commission on Radiation Protection recommends annual effective dose limits of 20 mSv for radiation workers and 1 mSv for the public (ICRP, 1991), with European airlines currently being requested to monitor the radiation exposure of flight personnel to cosmic radiation if their annual doses are expected to exceed 1 mSv.

“Low doses” of radiation have not been officially defined but for present purposes will be operationally defined as being ≤ 100 -200 mGy. For comparison purposes, the *BEIR VII Report* has defined low-dose radiation as in the range “near” zero up to about 100 mSv (NRC, 2006), whereas the report of the French Academy of Sciences and of the French Academy of Medicine has defined low doses as <100 mSv and very low doses as <10 mSv (Tubiana et al., 2005). The low-dose regime assumes especial importance since it encompasses the region where radiation-induced cancer becomes manifest. There is a fair amount of controversy regarding quantification of the dose threshold for radiation-induced cancer. The French Academies Report estimates a threshold value of 100 mSv from both human cancer epidemiology and experimental animal carcinogenicity. While the *BEIR VII Report* concurs that no data shows carcinogenic effects in humans below 100 mSv, it also concludes that doses of 10-20 mSv delivered to the human fetus are responsible for excess incidence of leukemia and solid tumors. This judgment is contrary to that stated in the 30TH Lauriston S. Taylor Lecture of the National Council on Radiation Protection and Measurements that while definitive quantitative

D. P. Hayes

risks for the oncogenic effects of ionizing radiation exposure to the embryo are not available, it appears that the embryo is not more sensitive than children (Brent, 2007). Some of the epidemiological foundations of the *BEIR VII Report*, including its human in-utero conclusions, have been criticized by Hayes (2008). A review paper has claimed that epidemiological data suggests increased cancer risk in humans for acute exposures of ~10-50 mSv and ~50-100 mSv for protracted exposures, with “reasonable” evidence for an increase in some cancer risks at doses above 5 mSv (Brenner et al., 2003). Aspects of that review paper have been critiqued by Tubiana et al. (2006), including its reliance on data purportedly showing low-dose in-utero effects.

RADIATION CLUSTERS: DEFINITIONAL BASIS

As a result of its non-homogenous energy deposition, ionizing radiation can produce a form of localized DNA and cell damage termed “cluster damage.” As reviewed by Ward (1994), clusters can also be produced by agents other than ionizing radiation. In addition, claims have also been made for possible clustered DNA damages induced in human cells by endogenous processes, albeit at low frequencies (Bennett et al. 2004). Clusters are also induced in DNA and in cells by radiomimetic anti-tumor drugs and at low frequencies by endogenous processes. The history of radiation-induced cluster damage has been reviewed by Goodhead (1989). As early as the 1940s it had been suggested that localized clusters of ionization were responsible for critical damage to DNA structure in eukaryotes (Lea, 1947). The biophysical analyses of Howard-Flanders (1958) indicated that the spatial extent of the critical clusters extended over a few nanometers; a result arising from the analysis itself, without any assumption as to the molecular nature of the target volumes or the relevance of DNA. Similar concepts were applied by Barendsen (1964) and later by Goodhead et al. (1980) to various mammalian cells, leading to the conclusion that critical damage was due to clustering of ionizations over dimensions of 2-10 nm. All these analyses were based on rather crude descriptions of ionizations and clusters; these being the best microscopic descriptions available at the time. What has later biophysical modeling revealed about the actual form of the cluster damage? In cells, about 30% of radiation-induced damage arises from direct energy deposition on the DNA and another, about 70% is formed from indirect energy deposition by hydroxyl radicals generated by radiolysis of water in the vicinity of the DNA (Ward, 1985). Within a cell, indirect actions occur over very short distances, of the order of a few nanometers (with ~2 nm being the width of the DNA double helix), because the diffusion distance of radicals is limited by their reactivity, i.e., radical scavenging action.

High concentrations of radiation-induced reactive oxygen species adjacent to DNA produce concomitant helix lesions. Note that “lesion” as

Nonproblematic risks from radiation damage clusters

used here designates an individual altered DNA site, whereas “damage” designates complex sites containing at least two lesions. (To place matters in perspective, if both double helix strands are broken simultaneously, a double-strand-break lesion is produced at this one damage site.) Ionizing radiation is known to produce a variety of individual non-clustered DNA lesions through direct and indirect effects, such as single strand breaks (SSBs), double strand breaks (DSBs), abasic sites (apurinic or apyrimidinic, AP), as well as DNA-DNA and DNA-protein cross-links along with a plethora of base modifications. However, due to the production of low-energy secondary electrons, X- and γ -rays impart energy and subsequent free radical products in clusters of lesions rather than in simple, uniform or random patterns. Clustered damages consist of multiple closely spaced lesions (strand breaks, oxidized base clusters, or abasic sites within a few helical turns). It has been estimated that energy deposition by a single radiation track near the DNA generates on average 2~5 ionizations in a 1~4 nm diameter which is within a helical turn on the DNA (Ward, 1988; Goodhead et al., 1993). Track-structure simulations have shown that a substantial portion of the radiation dose, up to ~50% for low-LET γ -rays, is deposited in the form of localized Bragg’s peak type clustered damage produced predominantly by low-energy (a few keV) high RBE secondary electrons near their terminal track-ends (Nikjoo and Goodhead, 1991). These low-energy “track end” electrons are similar to the photo- and Auger-electrons produced by ultrasoft X-rays. More highly localized energy deposition, whether from Auger electron emitting iodine-125 or high-LET radiation, causes more complex structures. While the dense ionization of high-LET radiation increases the complexity of clustered damage, there may be concomitant decrease in the number of damage clusters at high-LET.

Damage clusters encompassing several base pairs over a distance of a few nanometers are usually referred to as locally multiply damaged sites (LMDSs), a term and concept coined by Ward (1988). Clustered damage over kilobase-sized distances (owing to organization of the chromatin as a 30 nm fiber) is usually referred to as regionally multiply damaged sites (RMDSs). The LMDSs represent localized multiple lesions formed on either or both DNA stands within one or two helical turns and include SSBs, DSBs, base damage and complex contributions all within the damage cluster. Biophysical modeling indicates that from one to tens of radicals per site are formed; meaning that a LMDS could have tens of damaged moieties in it (Ward, 1991). These damages can be distributed across both strands over tens of base pairs, depending on size of the originating energy deposition event. As will be addressed in the next section, cells will be faced with a complexity of repair problems as a consequence of this variety of damage. The multiple lesions include both singular DSBs and complex DSBs (which may include DSBs⁺ with one double strand

D. P. Hayes

break accompanied by one or more single strand break on one strand only, DSBs⁺⁺ with at least two double strand breaks in the region of the hit of the DNA, etc.) The multiple localized lesions also include non-DSB clustered damage such as SSBs formed in close proximity to additional breaks and other closely-spaced lesions, e.g., oxidized purines, oxidized pyrimidines, abasic clusters such as an abasic site with another abasic site or an abasic site with an SSB. (The terms “abasic” or “oxybase” clusters refer to clusters containing at least one abasic site or one oxidized base, respectively.) Track-structure simulations of γ -ray induced damage indicate that nearly 30% of DSBs are of complex form solely by virtue of additional associated breaks. Inclusion of base damage increases the computed complexity to about 60%, a twofold increase in the frequencies of complex DSBs (Nikjoo et al., 1999).

RADIATION CLUSTERS: BASIS OF LABORATORY STUDIES

Laboratory studies of DNA clusters are both analytically and technically demanding. Their observations are at the cutting edge of current technology and fraught with technical difficulties (Goodhead, 1994; Gulston et al., 2002; Prise et al., 2001). As one specific example, present technology appears incapable of properly recognizing, distinguishing, and measuring more complex DSBs such as DSBs⁺ and DSBs⁺⁺, etc. (Sutherland et al., 2000). The theoretical basis, biochemical requirements, and practical aspects of current-day methods for detecting, identifying, and quantifying clustered DNA damage and repair have been reviewed by Sutherland et al. (2003). Bistranded clusters are studied, being defined as two or more lesions on opposing strands (strand breaks, oxidized bases or abasic sites within a few helical turns). Assessing the induction, repair and consequences of these clusters involves measurements of specific DNA lesions by methods independent of biological responses to such lesions. They have been quantified by separating DNA as a function of molecular size by gel electrophoresis, obtaining a quantitative image of the resulting distribution of DNA in the gel, and through number length analysis which provides high sensitivity and does not require any specific distribution of lesions with the DNA molecules. Cellular enzymes that cleave at DNA lesions provide a means of recognizing and measuring damages; for example, *Escherichia coli* Nfo protein (endonuclease IV) recognizes abasic clusters, *E. coli* Fpg protein recognizes oxypurine clusters, and *E. coli* Nth protein recognizes oxypyrimidine clusters. Of the different repair pathways base excision repair (BER) is generally believed to be the primary defense against clustered lesions other than double-strand breaks. Non-DSB clusters are detected and quantified in the BER pathway by cleavage with lesion-specific repair enzymes which produce *de novo* double-strand breaks (i.e., those pro-

Nonproblematic risks from radiation damage clusters

duced by enzyme cleavage, not directly by radiation). The new DNA breaks formed postirradiation are measured as an indication of clusters with their number corresponding to the number of radiation-induced clusters.

The local multiply damaged sites (LMDSs) initially predicted by biophysical modeling (Ward, 1988) have been subsequently observed (Prise et al., 1999; Milligan et al., 2000; Sutherland et al., 2000). Experimental studies have also shown that at low radiation doses a damage cluster can be produced by a single radiation “hit” (i.e., energy absorbed along a particle track caused by ionizing radiation in tissue (ICRU, 1983)), thus indicating that two independent hits are not required for cluster induction (Sutherland et al., 2001). Enzymatic experimental methods indicate that complex non-DSB damage (e.g., abasic clusters containing abasic sites, clusters with oxidized pyrimidines, clusters with oxidized purines, et al.) induced in mammalian cells by γ -radiation and X-rays constitute the majority of multi-lesion damage, with yields some four to eight times that of prompt DSBs. These estimates have been separately reported by Sutherland et al. (2000 and 2002) and Gulston et al. (2002) who have also enumerated specific reasons why clustered damage may even have been experimentally underestimated. Ward (2000) reports on observations indicating that DSBs are only a minor fraction (~12%) of the LMDSs produced by ionizing radiation, that if two lesions on the same strand of the DNA are considered to be LMDSs then DSBs will constitute only about 6% of the total LMDSs, and that proteins involved in repair may bind to both DSBs as well as to these complex lesions. Similar ratios of non-DSB clustered DNA damage following high-LET charged particle radiation have also been reported (Prise et al. 1999; Sutherland et al., 2000). However, it should be noted that it has been claimed that due to spurious experimental oxidation the amount of non-DSB cluster damage (LMDSs composed of oxidized purines or pyrimidines) may have been previously overestimated, and in actual fact a large portion of LMDSs may consist of difficult to repair and mostly lethal complex DSBs associated with oxidative damage (Boucher et al., 2004, 2006).

The evidence arising from the published literature that has been presented here may lead one to the conclusion that clustered damage has already been reliably determined in living cells, and even in unirradiated control cultures. But as noted in the introduction and in parts of the preceding discussion, laboratory studies of DNA clusters are both analytically and technically demanding and consequently fraught with uncertainties. While significant progress has been made in these studies, caveats still remain. Complicating factors include the fact that damage clusters differ enormously in composition, that at present there is no method available to adequately quantify these clusters in living cells, that the biological consequences of clustered damage are far from being fully under-

D. P. Hayes

stood, and that reports of the presence of endogenous clusters have not as yet been confirmed. Thus more hard scientific data are needed to provide quantitative risk evaluations in the low dose range.

REPAIR VIS-À-VIS RADIATION CLUSTERS

It would be expected that cellular recognition and repair systems are likely to see clustered DNA lesions as substantially different from non-clustered lesions. While isolated oxidized bases and abasic sites in DNA can be removed effectively by a panoply of lesion-recognizing glycosylases and endonucleases (Wallace, 1998), the different types of bistranded or uninstranded clustered DNA lesions conceptually appear to challenge cell repair. It seems reasonable to hypothesize that cells will be best able to repair more minor DNA damage, and that perfect repair will concomitantly become less probable for more complex damage. For example, complex double-stranded breaks involving clusters of ionizations and of DNA-damaging events would be expected to be more refractory to repair than “simple” double-stranded discontinuities because both strands of the complex helix are locally damaged. These concepts form the basis of proposals that the more complex cluster damage would be less efficiently handled by the cell’s battery of repair enzymes, and that the cell’s attempted repair of clustered DNA damage would result in unrepairability or in some form of misrepair arising from loss or distortion of coding information. Unlike damage to a single strand of the DNA duplex, it would be expected that a proportion of double-stranded lesions, perhaps the component represented by LMDs, would result in the loss or distortion of coding from both strands. Such losses or distortion would make correct repair difficult in mammalian cells.

The concept that multiple lesions close together within damage cluster sites present repair problems, i.e., they would be misrepaired or less readily repaired than when present as isolated lesions, and the corollaries that repair becomes more difficult and perfect repair less probable with damage complexity and that increasing number of lesions per damage site would concomitantly mark decreased enzymatic repair, have been hypothesized by Goodhead et al. (1980, 1993), Ward (1981, 1991) and Goodhead (1994). Some of the reasons which have been advanced to support this hypothesis include the following: clustered DNA damage is likely to be more difficult to repair since repair enzymes that recognize different simple types or components of lesions may interfere with each other, additional damage sites may interfere with the recognition and binding of repair enzymes to the DNA, repair started on one type of damage may make matters worse when another site of damage is nearby, and that clustered damage may effectively destroy the local coding sequence (Chaudhry and Weinfeld, 1997; Harrison et al., 1998).

Nonproblematic risks from radiation damage clusters

Later laboratory work has lent credence to previously hypothesized repair problems in showing inhibition of the processing of lesions within clustered DNA damage, and that the different types of bistranded or uninstranded clustered DNA lesions appear to be the most challenging lesions for the cell to repair. Evidence is accumulating that the processing of lesions within clustered damage sites by base excision repair (BER) is compromised relative to individual lesions (Chaudhry and Weinfeld, 1997), whereas increasing the inter-lesion gap allows DNA glycosylases to excise and initiate the BER pathway (Harrison et al., 1998, 1999). Both *in vitro* measurements of repair enzymes acting on synthetic oligonucleotides containing defined clusters (Harrison et al., 1999; David-Cordonnier et al., 2000; Georgakilas et al. 2002), and studies of abasic cluster processing in repair-proficient human cells (Georgakilas et al., 2004) indicate that many clusters are refractory to repair. For example, studies of the efficiency of different repair enzymes in cleaving oligonucleotides duplexes containing closely spaced DNA lesions showed that closely spaced AP sites, oxidized bases or single-strand breaks (SSBs) reduced or eliminated recognition and/or cleavage. *In vitro* studies of the repair of complex DSBs with combinations of base damage and strand breaks have shown inhibition of the action of DNA lesion-recognizing proteins due to perturbation of the DNA helix structure flanking such damage sites (David-Cordonnier et al., 2001). In addition, *in vitro* observations support the notion that non-DSB γ -radiation-induced damage (i.e., base damage and SSBs) upstream from an enzyme-induced DSB is a potent inhibitor of human nonhomologous end joining (Pastwa et al., 2003).

As has been noted, it was initially believed that the postulated lack of repair or misrepair in clusters would be responsible for extremely problematic concomitant DNA damage. For example, Goodhead and Nikjoo (1997) have stated that if the more complex damages are repaired less efficiently by the cell (i.e., biologically more “severe”), then these might well be the biologically dominant classes of radiation damages despite the very much larger yields of simple damage from single ionizations. The UNSCEAR Report 2000 (United Nations, 2000) has stated that a proportion of double-strand lesions, perhaps that component represented by LMDSs, will result in the loss of DNA coding from both strands, and that such losses are inherently difficult to repair correctly and that misrepair of such DNA double-strand lesions is the critical factor underlying the principal hallmarks of stable mutations induced by ionizing radiation of various qualities (United Nations, 2000).

Recent work has questioned these concerns in pointing out that at low radiation doses and/or dose-rates clustered damages might prove efficacious! The basis for these conjectures is that when the dose and/or dose-rate are low, radiation damage sensors are not activated, there is lit-

D. P. Hayes

tle or no repair (in strong contrast at higher dose and dose-rates) resulting in permanent cell cycle arrest, and that damaged cells with complex lesions are eliminated either by apoptotic or mitotic death (Joiner et al., 2001; Marples et al., 2003). Apoptosis eliminates damaged or misrepaired cells and varies by dose and dose rate, while mitotic death occurs during mitosis when lesions have not been repaired due to lack of activation of cell defense mechanisms. Radford (2002) has suggested that the difference in lethal effectiveness between DNA-incorporated ^3H and ^{125}I decays reflects the relative efficiency with which these radionuclides induce complex DNA damage, leading to the hypothesis that cell killing is attributable to complex DSBs (therein defined as two single strand breaks plus two or more base damages or strand breaks) and that simple DSBs (therein defined as two single-strand breaks) are non-lethal. Clusters now assume a *dues ex machina* role in that their very complexity which abrogates repair is now responsible for their eventual demise and concomitant elimination as a risk factor!

Germane to this discussion are modern transcription analyses of cellular genes using DNA microarray technology. They reveal that irradiation at levels below that causing detectable mutational or lethal biological effects can change intracellular signaling without modifying the genome and either activate or inhibit numerous genes involved in general metabolism and in defense against ionizing radiation (Mercier et al., 2004; Yin et al., 2003). The sets of genes that are either activated or inhibited vary with dose and dose rate, indicating not one defense system but several. Such mechanisms bring into play defenses at low doses, which make it possible to reduce or prevent potentially harmful radiation effects. In support of this proposal is the fact that damage signaling redundancy at high doses not present at low doses has been invoked to explain differences in repair efficiency (Fernandez-Capetillo et al., 2002; Mochan et al., 2003).

A critical question that arises is why a postulated repair system has evolved which requires activation above a DNA damage threshold. One possibility is that it is beneficial for the organism to allow small numbers of damaged cells to die, rather than to risk mutations through repair and survival (Marples, 2004). When only a few cells are damaged, this elimination strategy would appear to be optimal, since repair systems would be expected to be error-prone (especially for the more complex cluster damage) leading to the emergence of precancerous and subsequently cancerous cells. This explanation predicts that the presence of a few unrepaired damage sites does not increase the risk of mutation, but rather serves to identify damaged cells for elimination from the population. The bottom line is that lack of repair below the damage threshold instead of being deleterious proves to be beneficial by either eliminating or at least reducing the numbers of radiation-induced clusters.

Nonproblematic risks from radiation damage clusters

It should be noted that in addition to the potential for lethal cluster damage there have also been reports of the possibility of mutagenic damage induced by ionizing radiation. Some in-vitro results on the reparability of clustered damaged sites in different configurations on oligonucleotides may indicate the generation of short single stranded regions carrying unrepaired base damage, later filled by repair synthesis, and leading to point mutations (Eot-Houllier et al., 2007). (Although it should be noted that there is uncertainty whether those specific clustered legions are actually formed after ionizing radiation.) In addition, some results obtained after overexpression of repair enzymes in living cells have been interpreted as indicating that attempted repair of radiation damage, presumably at clustered damage sites, leads to both cytotoxic and mutagenic effects. (Yang et al., 2004; Yang et al., 2006).

RADIATION CLUSTERS: LABORATORY OBSERVATIONS

This section will provide a summary of experimental laboratory observations of pertinence to radiation-induced clusters, and in particular those lending credence to the proposals presented in the preceding section that radiation below some threshold level triggers processes that bypass repair and cause precancerous and/or cancerous cells to be eliminated. Laboratory studies to be reviewed include γ -H2AX phosphorylation, hyperradiosensitivity (HRS) and increased radioresistance (IRR), neoplastic transformations, and pKZ1 recombination mutation assays.

 γ -H2AX Phosphorylation Studies

Sensitive mechanisms have recently evolved for detecting and monitoring the consequences of radiation-induced cell damage. Among these are the use of a fluorescent antibody specific for the local formation of the phosphorylated histone γ -H2AX which provides a useful and reliable biomarker for the quantification of DNA double-strand breaks. (Phosphorylation is the introduction of a phosphate group into an organic molecule and is catalyzed by various specific protein kinases, with histones being a form of nuclear proteins.) Exposure to ionizing radiation results in the rapid phosphorylation of a special form of histone 2A, denoted H2AX, that is part of 10% of all nucleosomes in the cell (Rogakou et al., 1998). Exposure produces discrete nuclear foci at the site of DSB damage, the γ -H2AX foci, which act as a “molecular beacon” to recruit DNA-repair factors to the DSB site. The potential to detect a single focus within the nucleus makes this one of the most sensitive methods currently available for detecting unrepaired DSBs in cells, enabling detection over the important 1-10 mGy dose range (Rothkamm and Löbrich, 2003). The response to a single DSB is both rapid (within minutes) and highly amplified; and initially involves the phosphorylation of

D. P. Hayes

hundred to thousands of histone H2AX molecules surrounding the DSB, forming foci in interphase nuclei as well as megabase chromatin domains surrounding the DNA lesion (chromatin being a complex structure formed by chromosomal DNA and histones). Although the kinetics of total cellular histone H2AX phosphorylation after irradiation have been characterized, the phosphorylation kinetics of individual γ -H2AX foci and the exact mechanisms by which they are induced still remain to be completely elucidated. However, the γ -H2AX foci are certainly essential to the recruitment to the site of a number of proteins involved in DNA repair and with subsequent activation of downstream functions such as p53 and checkpoint kinases resulting in changes in repair, cell cycle progression, and cell death induction. Accumulated evidence indicates that the focus formation of γ -H2AX precedes and signals the involvement of repair enzymes involved in the processes of homologous recombination (HR) and non-homologous end-joining (NHEJ) repair of DNA double-strand-breaks, and that dephosphorylation coincides with DNA repair. Several of the proteins involved in the sensing, signaling and repair of DSB have been identified and are known to co-localize with γ -H2AX following DNA double-strand induction in mammalian cells. After induction of DSBs by ionizing radiation, H2AX is thought to be phosphorylated at a particular amino acid (the serine at position 139 in the protein) by the activated protein kinase ATM, the product of the ATM gene which is mutated in the human disease ataxia-telangiectasia. Activation of the ATM kinase seems to be an initiating event in cellular response to radiation. The related DNA-activated protein kinases, DNA-PKs (DNA-dependent protein kinase) and ATR (ATM and Rad3-related) may serve as backups at later times (Averbeck, et al. 2006; Bakkenist and Kastan, 2003; Bartek and Lukas, 2003; Stiff et al., 2004). The signaling of clustered lesions is still unknown.

One of the most important γ -H2AX foci study yet published is that of Rothkamm and Löbrich (2003). For stationary nondividing primary human fibroblasts they showed that γ -H2AX foci can be detected after single x-ray doses as low as 1 mGy and that the number of DSBs formed, as measured by the number of foci formed, is linear with dose from 1 mGy to 100 Gy. Importantly, this *in vitro* study showed a lack of DSB repair at low-dose levels. A damage threshold level of ~ 1 mGy was reported above which repair mechanisms operate efficiently, but at and below which repair is either impaired or inoperable. This threshold dose level corresponds to a dose at which a human fibroblast nucleus is traversed, on average, by approximately one electron track, so that further lowering the dose will not decrease the actual amount of damage received per single cell but will merely lower the fraction of cells hit by radiation particles (ICRU, 1983). In addition, it was found that if cells were allowed to proliferate after ~ 1 mGy irradiation, DSB levels decreased to that of unirra-

Nonproblematic risks from radiation damage clusters

diated cell cultures with substantially more apoptotic and micronucleated cells than the unirradiated controls. These results are in contrast to current models of risk assessment that assume that cellular responses are equally efficient at low and high doses. Rothkamm and Löbrich (2003) specifically proposed that the observed lack of DSB repair at very low radiation doses does not increase the carcinogenicity risk, but rather represents a protective biological mechanism to reduce it. Instead of repairing a DSB in a particular cell with the risk of causing genetic alterations, they proposed that it could be beneficial for an organism to remove the damaged cell and replace it by the division of an undamaged neighboring cell when only a small fraction of cells carry a DSB, and that repair is necessary only at higher radiation damage levels. Of course this is the paradigm that had been previously proposed by Joiner et al. (2001). A γ -H2AX foci study coauthored by one of the Rothkamm and Löbrich (2003) principals reported on *in vivo* formation and repair of normal lymphocytes DSBs after computed tomography (CT) examinations of humans (Löbrich et al., 2005). In contrast to the *in vitro* studies of Rothkamm and Löbrich (2003), it was found with one exception (a subject with repair defect) that DSBs were completely repaired to background levels. But the lowest dose studied was 5 mGy, well above the ~ 1 mGy *in vitro* repair threshold previously reported by Rothkamm and Löbrich (2003). Among studies of related interest are those of cell proliferation. Anoopkumar-Dukie et al. (2005) reported that diagnostic X-rays over the range 7.5-55.1 mGy significantly impaired subsequent cell proliferation, whereas cell response at 3.5 mGy was not distinguishable from controls. While the existence of a dose threshold could not be established, they stated that if it existed it would be below 7.5 mGy.

Rothkamm and Löbrich's (2003) reporting that single low radiation doses delivered at high dose rate (HDR) can evade early DNA damage-detection mechanisms raises the important question of whether low dose rate (LDR) exposures can cause similar evasion. This question has been answered in the affirmative by Collis et al. (2004) who compared the response of human cells exposed to equivalent doses of ionizing radiation delivered at either HDR or LDR. After delivery of 2 Gy γ -irradiation dose at a low dose rate, both ATM (a protein critical in signaling from DSBs) and γ -H2AX foci formation were found to be absent, whereas they were clearly activated by the same dose delivered at a slightly higher dose rate, as well as at an even higher dose rate. Collis et al. (2004) argued that the reduced activation of ATM and H2AX is not simply a result of reversal by dephosphorylation during the protracted time it takes to deliver the dose at LDR. They likewise argued that the increased cytotoxicity following low-level DNA damage may represent a protective mechanism which enables the cell to avoid mutations arising from error-prone DNA repair. Complementary findings have been reported by Ishizaki et al. (2004) who

D. P. Hayes

examined immortalized confluent normal human fibroblasts. They reported that HDR exposure induced significant levels of γ -H2AX foci whereas chronic LDR induced only a few such foci, and that p53 phosphorylation of a particular amino acid, the serine at position 15 in the protein (which is largely mediated by ATM) was abrogated at LDR vis-à-vis HDR.

The lack of DNA damage signaling following LDR exposure associated with increased human cell lines killing reported by Collis et al. (2004) is an effect previously termed the “inverse dose rate effect” (Mitchell and Joiner, 2002; Mitchell et al., 2002). Collis et al. (2004) ascribed the increased cell death of the inverse dose rate effect to be a consequence of reduced repair or a reduction in initiation of repair signals, and that it represents a default mechanism by which the cells minimize the likelihood of passing on promutagenic lesions to its progeny. Similar inverse dose rate effects are also known to exist in the production of germ-line and somatic mutations, genetic recombination, chromosomal translocation, cell inactivation and lethality, and human leukemogenesis (Vilenchik and Knudson, 2000 and 2006), which Collis et al. (2004) proposed may likewise be a consequence of ineffective activation of repair mechanisms.

In summary, the study of Collis et al. (2004) clearly indicates that cellular radiation response depends on dose rate; with low rate evasion of “cellular radar” damage detection marked by concomitant changes in DNA damage signaling and repair activation resulting in increased cell lethality. The data of both Rothkamm and Löbrich (2003) in concert with that of Collis et al. (2004) clearly show that when the dose or dose rate is low the radiation damage sensors are either abrogated or downgraded with consequent changes in repair and elimination of damaged cells.

Hyperradiosensitivity (HRS) and Increased Radioresistance (IRR) Studies

For most cell types mortality is very high (per unit dose) from irradiation onset through the first few hundred mGy and reaches a nadir before reversing course. These mortality variations indicate that the cellular defense mechanisms against lethality, which initially show little efficacy, become more effective with increased irradiation. Hyper-radiosensitivity (HRS) is the term for the phenomenon in which cells die from either small single doses of ionizing radiation, or from radiation delivered at low dose rates with the same total dose. Increased radioresistance (IRR) is the term for the phenomenon whereby these cells become more resistant to lethality from either larger single doses or rates of radiation (Joiner et al., 2001; Mitchell et al., 2002). The phenomenon of induced radioresistance is not unexpected in mammalian cells in light of historical reports in lower organisms (Joiner et al., 1996), and from observations assessing the radiosensitivity of insect cells (Koval, 1988). The

Nonproblematic risks from radiation damage clusters

interplay of HRS and IRR can lead to striking results in which a lower dose or dose rate leads to greater cell killing than a higher dose or dose rate. Typically, most mammalian cell lines exhibit hyperradiosensitivity to low radiation doses that is not predicted by back-extrapolating the cell survival response from higher doses. As dose is increased there is increased radioresistance until at doses beyond about 1 Gy radioresistance is maximal and the cell survival curve follows the usual conventional downward-bending with increased dose. Circa year 2004, HRS and IRR have been characterized in over 40 X-irradiated human cells lines using different qualities and biological endpoints, and even after acute dose rate proton and pi-meson irradiation and after high-LET neutrons given at a low dose rate (Marples, 2004). These data demonstrate that HRS is a phenomenon universal at low levels (and possibly rates) of radiation in mammalian cells irrespective of incident radiation LET. Consequently, it has been postulated that HRS is the default survival response of cells to radiation injury for doses less than $\sim 200\text{--}300$ mGy (Joiner et al., 2001; Marples et al., 2003). As pointed out by Bonner (2004), this biphasic HRS / IRR dose response may be an example of hormesis. Hormesis can be defined as a dose-response relationship phenomenon characterized by low-dose stimulation and high-dose suppression of protective processes, and is exemplified in toxicology and nutrition when beneficial effects are stimulated by a low exposure of an agent otherwise considered detrimental (i.e., toxic) at higher exposure (Hayes, 2007).

A number of hypotheses have been proposed to explain HRS. The mechanism(s) underlying HRS appear to be related to changes in repair fidelity or efficiency in a dose or damage dependent manner (Joiner et al., 2001). One explanation is that the HRS response can be attributed to the death of a fraction of G2-phase cells that evade cell cycle arrest processes and therefore enter mitosis with unrepaired DNA damage and die by apoptosis (Marples et al., 2003 and 2004). Studies have established relationships between apoptosis and hyperradiosensitivity, e.g., suggesting that HRS is likely a measure of the apoptosis of radiation-damaged G2-phase cells that evade early G2-phase checkpoint arrest (Krueger et al., 2007), and that HRS is associated with p53-dependent apoptosis (Enns et al., 2004).

The balance of evidence suggest that the activation of DNA repair per se, or process(es) associated with the repair of DNA damage, are important for overcoming HRS and for the development of IRR, and concomitantly that HRS is a measure of radiation sensitivity in the absence of fully functional repair. Marples and Joiner (2000) have shown IRR to be correlated with induction of DNA repair. Marples (2004) reported on various other experiments that support the concept that a threshold amount of DNA damage needs to occur to overcome low-dose hyperradiosensitivity.

D. P. Hayes

ity and induce increased radioresistance. He specifically cited the γ -H2AX foci data of Rothkamm and Löbrich (2003) and whole cell studies using dual radiation exposures as supporting the notion that repair factors are activated only after the number of DSBs exceeds a putative threshold, whereas repair is strongly compromised at or below the threshold number of events. Marples (2004) has also linked HRS dose-rate studies to repair, suggesting that the failure of the low dose rates to induce an increased radiation-resistance response may indicate that either the accrual of radiation damage or the rate of damage receipt were at rates below that necessary to induce the cell repair mechanism responsible for increased radioresistance.

Hyperradiosensitivity is associated with both the adaptive response (classically thought of as the induction of some sort of protective mechanism, e.g., DNA repair) as well as with the inverse dose-rate effect. The requirement to exceed a threshold level of radiation injury for the full induction of repair processes was established by what has become known as the adaptive response (Bonner, 2004; Marples, 2004). Adaptive response following low dose “priming” radiation is typically demonstrated by the reduced effect of a subsequent high “challenge” dose and has been demonstrated both *in vitro* and *in vivo* using a variety of endpoints. These responses temporarily up-regulate defenses against, repair of, and removal of damages after a triggering event (Feinendegen, 2005; Feinendegen and Neumann, 2006). In experiments designed to measure the effect of various preexposure doses on the response to a subsequent test dose in the HRS region of cell lines exhibiting HRS/IRR, it was found that HRS can be decreased or eliminated in the test dose population if cells are “primed” for DNA damage repair by preexposure radiation doses which exceed a DNA damage threshold level. It appears that HRS/IRR phenomenon is a manifestation of the same underlying mechanism that determines the adaptive response and likewise depends on the amount and rate of DNA repair (Joiner et al., 1996). It has also been pointed out that those HRS/IRR studies which indicate increased cell killing at low dose rates of continuous exposure are just another manifestation of the inverse dose rate effect with both processes sharing the same endogenic radioprotection biochemical mechanisms (Mitchell et al., 2002; Leonard, 2007).

Neoplastic Transformation Studies

Neoplastic transformations are the radiation-induced conversion of cells in culture from a non-tumorigenic to a tumorigenic phenotype. Such phenotypes are identified by whether or not cells will grow tumors following implantation into suitable host animals (Redpath, 2006a). While these cells are not normal in that they are immortal and do not

Nonproblematic risks from radiation damage clusters

exhibit contact-inhibited growth, they nevertheless are non-tumorigenic and as such should be regarded as pre-neoplastic cells. The study of radiation effects on such cells is of importance since healthy humans harbor pre-neoplastic tissue. The only present-day quantitative transformation assay that is human cell-based is the HeLa x human skin fibroblast human hybrid cells assay used by Redpath and associates. For low-LET radiation, *in vitro* neoplastic transformations can be suppressed to levels below that seen spontaneously. Redpath and Elmore (2007) state that for dose rates of 30-3000 mGy/min the threshold dose for the induction of neoplastic transformations is around 100-200 mGy, while for lower dose rates (<0.5 mGy/min) it occurs at doses >1000 mGy. Neoplastic transformation suppression and eventual initiation over such a large range indicates involvement of multiple mechanisms whose relative contribution may vary with dose: at lowest doses the killing of a subpopulation of cells already destined to become neoplastically transformed, while at somewhat higher doses the induction of DNA repair (Redpath, 2005). The role of DNA repair in neoplastic transformations has been reported at 50 mGy, but not at the lower dose of 5 mGy (Pant et al., 2003). Neoplastic transformations have also been linked to cellular hyperradiosensitivity and induced repair by Redpath et al. (2003b) and in supporting, at least in a qualitative sense, the results and explanations of the phosphorylation studies of Rothkamm and Löbrich (2003). In addition, Portess et al. (2007) have reported data showing that low-dose irradiation of nontransformed cells stimulates the selective removal of precancerous cells at doses as low as 2 mGy γ -rays and 0.29 mGy α -particles, with the radiation-stimulation effect saturating at doses of 50 mGy γ -rays and 25 mGy α -particles. They likewise note that this selective removal of precancerous cells may represent a natural anti-cancer mechanism stimulated by low doses of ionizing radiation. While they attribute this selective removal to intercellular apoptosis, they note that it might likewise be explained by the transformation frequency reduction proposed by Redpath et al. (2001).

The radiation-induction of cancer in humans is dependent on many factors, both genetic and epigenetic, that are not possible to duplicate in cells *in vitro*. Therefore, *a priori*, one would not expect that studies of pre-neoplastic cells *in vitro* to have a direct quantitative link to risk estimates in human populations. Despite these caveats, Redpath (2004) and Redpath and Elmore (2007) report that relative *in vitro* risk estimates over the range 1 to 1000 mGy data agreed “surprisingly” well with those for radiation-induced breast cancer and leukemia in humans at doses >100-200 mGy. This has been shown both for fluoroscopic energy X-rays of the type used in diagnostic radiology (Redpath et al., 2003a), and for mammography energy X-rays (Ko et al., 2006). These results are important since medical X-rays are the preponderant component of man-made ionizing radiation. In addition, Redpath and Elmore (2007) have reported

D. P. Hayes

that the two experimental neoplastic transformation monitoring systems (the C3H10T1/2 and the human cell-based HeLa x skin fibroblast human hybrid cell assays) have both demonstrated hormetic responses at low doses of low LET radiation. For example, neoplastic transformations of human hybrid cells by Cs-137 gamma rays and 60 kVp X-rays both display the J-shaped dose-response curves characteristic of hormesis (Redpath et al., 2003a; Redpath, 2006b).

pKZ1 Recombination Mutation Assay Studies

Rearrangement and loss of genetic material are common mutations in cancer and can result from a process called recombination. Somatic intrachromosomal recombination, which leads to chromosomal deletions and inversions, is an important mutational mechanism. Chromosomal inversions are a common mutation in cancer and can be regarded as a surrogate measure for cancer. The action of DNA damaging agents can be studied using the pKZ1 transgenic mouse as a mutation assay with somatic intrachromosomal inversions as the mutation end-point. The pKZ1 mice have a marker gene that can lead to production of blue-color stained cells in which a chromosomal inversion has occurred. *In vivo* pKZ1 transgene assays have proved to be very sensitive for detecting changes in chromosomal inversions in lymphoid tissues in response to low doses of X-ray and various chemical DNA damaging agents. All published pKZ1 inversion assay studies have been by Sykes and colleagues, and their bases have been detailed by Sykes et al. (2006a, b).

Single acute whole body X-radiation has been found to cause significant inversion frequency changes in both the spleen and prostate of pKZ1 mice (with inversion frequency being the ratio of treated / control). Radiation doses over approximately the 1-10 mGy range caused decreases below endogenous inversion frequency, while doses above approximately 100 mGy and over approximately the 0.005-0.01 mGy range caused increases above endogenous levels (Hooker et al., 2004; Zeng et al., 2006). The dose response curves were similar in both spleen and prostate, suggesting that the pKZ1 assay measures a fundamental response to DNA damage which is independent of tissue type, and that there is increased mutagenesis at doses <0.01 mGy and >100 mGy vis-à-vis doses at 1-10 mGy. While the precise mechanism(s) behind the dose-response is not presently known (Sykes et al., 2006a), it has been proposed that observations <0.01 mGy can be explained by low-fidelity, error-prone DNA repair/apoptosis, and in the 1-10 mGy region by p53-dependent, high-fidelity DNA repair/apoptosis in conjunction with a presumed p53-independent protective apoptosis-mediated process (Scott et al., 2007). An alternative explanation offered here is repair down-regulation throughout both dose regions, but with appreciable apoptosis down-reg-

Nonproblematic risks from radiation damage clusters

ulation only at the lower dose regime. Radiation doses <0.01 mGy (sometimes called the ultra-low-dose zone) will not be given further consideration as risk factors in this report since background radiation over a few weeks or longer may exceed this dose zone.

Redpath (2005) states that *in vivo* pKZ1 recombination mutation assay results in the 1-10 mGy region are largely compatible with *in vitro* neoplastic transformation studies, and as already noted neoplastic transformations in this dose range have been explained by repair diminution.

Summary of Laboratory Observations

The laboratory results which have been reviewed in this section and considered as risk factors lend credence to the concept of the necessity for thresholds to be exceeded for repair processes to take place and for below which repair is circumvented and precancerous and/or cancerous cells are eliminated. It should be noted that those reporting these experimental studies have not linked their observations to that of cluster formation which is here being proposed as a critical component in triggering cell elimination.

The reported quantitative repair thresholds of relevance to risk consideration include the following: 1 mGy from H2AX phosphorylation studies, 200-300 mGy from HRS/IRR studies, 100-200 mGy from high dose-rate neoplastic transformation studies, and >100 mGy from chromosomal inversion studies (pKZ1 recombination mutation assays).

DISCUSSION AND CONCLUSIONS

Radiation-induced DNA damage clusters have been described on the basis of both their biophysical modeling and laboratory observations. Previous suggestions and perceived wisdom have been that such clusters pose a very serious threat of biological damage due to intractability of their repair. In actuality, at least in the low-dose and low-LET region, this abrogation or downgrading of repair proves to be beneficial since it allows elimination of damage clusters through various processes, e.g., apoptosis, mitosis. Clusters may be a *sine qua non* and assume a *deus ex machina* role in that their very complexity which hinders or defeats repair is now responsible for their eventual demise and concomitant elimination or reduction as risk factors. Laboratory observational evidence has been presented which supports the paradigm that repair occurs above some threshold dose level below which clusters are either reduced or eliminated. The bottom line is that repair inhibition/cessation below some damage threshold proves to be beneficial instead of deleterious and contributes to non-problematic risks. A previous discussion of the failure of radiation-induced clusters to induce significant problematic DNA damage was relegated to making comparisons vis-à-vis dietary micronutrient deficiencies (Hayes, 2006).

D. P. Hayes

The paradigm that radiation-induced clusters may actually reduce risk in the low-dose region is consistent with the fact that low-dose environmental level epidemiological studies have not detected deleterious radiation effects in humans. This statement regarding dearth of low-dose environment risks is both seconded and enlarged upon by the following declaration of NCRP No. 136: “it is important to note that the rates of cancer in most populations exposed to low-level radiation have not been found to be detectably increased and in most cases the rates have appeared to be decreased” (NCRP, 2001). These statements and conclusions have been verified, updated and expanded upon by Hayes (2008) in his critical evaluation of the *BEIR VII Report*.

ACKNOWLEDGMENTS

I again wish to acknowledge the stakhanovite efforts and services of the staff of the William Hallock Park Memorial Public Health Library of the New York City Department of Health and Mental Hygiene. I also wish to acknowledge the valued suggestions of two unidentified reviewers which were incorporated into the revised manuscript.

REFERENCES

- Anoopkumar-Dukie S, McMahon A, Allshire A, and Conere TJ. 2005. Further evidence for biological effects resulting from ionizing radiation doses in the diagnostic X-ray range. *Br J Radiol* 78: 335-337.
- Averbeck D, Testard I, and Boucher D. 2006. Changing views on ionising radiation-induced cellular effects. *Int J Radiation* 3: 117-134.
- Bakkenist CJ and Kastan MB. 2003. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* 421(6922): 499-506.
- Bardensen GW. 1964. Impairment of the proliferative capacity of human cells in culture by alpha-particles with differing linear-energy transfer. *Int J Radiat Biol Relat Stud Phys Chem Med* 8:453-466.
- Bartek J and Lukas J. 2003. DNA repair: Damage alert. *Nature* 421(6922): 486-488.
- Bennett PV, Cintron NS, Gros L, Laval J, and Sutherland BM. 2004. Are endogenous clustered DNA damages induced in human cells? *Free Radic Biol Med* 4: 488-499.
- Bonner W M. 2004. Phenomena leading to cell survival values which deviate from linear-quadratic models. *Mutat Res* 568: 33-39.
- Boucher D, Hendo J, and Averbeck D. 2004. Increased repair of gamma-induced DNA double-strand breaks at lower dose-rate in CHO cells. *Can J Physiol Pharmacol* 82: 125-132.
- Boucher D, Testard I, and Averbeck D. 2006. Low levels of clustered oxidative DNA damage induced in low and high LET irradiation in mammalian cells. *Radiat Environ Biophys* 45: 267-276.
- Brenner DJ, Doll R, Goodhead DT, Hall EJ, Land CE, Little JB, Lubin JH, Preston DL, Preston RJ, Puskin JS, Ron E, Sachs RK, Samet JM, Setlow RB, and Zaider M. 2003. Cancer risk attributable to low doses of ionizing radiation: assessing what we really know. *Proc Natl Acad Sci USA* 100: 13761-13766.
- Brent RL. 2007. Lauriston S. Taylor Lecture: Fifty years of scientific research: the importance of scholarship and the influence of politics and controversy. *Health Phys* 93: 348-379.
- Chaudhry KM and Weinfeld M. 1997. Reactivity of human apurinic/apyrimidinic endonuclease and *Escherichia coli* exonuclease III with bistranded abasic sites in DNA. *J Biol Chem* 272: 15650-15655.
- Collis SJ, Schwaninger JM, Ntambi AJ, Keller TW, Nelson WG, Dillehay LE, and Deweese TL. 2004. Evasion of early cellular response mechanisms following low level radiation-induced DNA damage. *J Biol Chem* 279: 49624-49632.

Nonproblematic risks from radiation damage clusters

- David-Cordonnier M-H, Laval J, and O'Neill P. 2000. Clustered DNA damage: influence on damage excision by XRS5 nuclear extracts and *Escherichia coli* Nth and Fpg proteins. *J Biol Chem* 275: 11865-11873.
- David-Cordonnier M-H, Laval J, and O'Neill P. 2001 Recognition and kinetics for excision of a base lesion within clustered DNA damage by the *Escherichia coli* proteins Fpg and Nth. *Biochemistry* 40: 5738-5746.
- Enns L, Bogen KT, Wizniak J, Murtha AD, and Weinfeld M. 2004. Low-dose radiation hypersensitivity is associated with p53-dependent apoptosis. *Mol Cancer Res* 2: 557-566.
- Eot-Houllier G, Gonera M, Gasparutto D, Giustranti C, and Sage E. 2007. Interplay between DNA N-glycosylases/AP lyases at multiply damaged sites and biological consequences. *Nucleic Acids Res* 35: 3355-3366.
- Federal Register. 1987. Radiation protection guidance to federal agencies for occupation exposure. *Federal Register* 52: 2822-2834.
- Feinendegen MD. 2005. Evidence of beneficial low level radiation effects and radiation hormesis. *Brit J Radiology* 78: 3-7.
- Feinendegen MD and Neumann RD. 2006. The issue of risk in complex adaptive systems: the case of low-dose radiation induced cancer. *Hum Exp Toxicol* 25: 11- 17.
- Fernandez-Capetillo O, Chen HT, Celeste A, Ward I, Romanienko PJ, Morales JC, Naka K, Xia Z, Camerini-Otero RD, Motoyama N, Carpenter PB, Bonner WM, Chen J, and Nussenzweig A. 2002. DNA damage-induced G2-M checkpoint activation by histone H2AX and 53PB1. *Nat Cell Biol* 4: 993-997.
- Georgakilas AG, Bennett PV, and Sutherland BM. 2002. High efficiency detection of bistranded abasic clusters in DNA by putrescine. *Nucleic Acids Res* 30: 2800- 2808.
- Georgakilas AG, Bennett PV, Wilson JW, and Sutherland BM. 2004. Processing of bistranded abasic DNA clusters in γ -irradiated human hematopoietic cells. *Nucleic Acids Res* 32: 5609-5260.
- Goodhead DT. 1989. The initial physical damage produced by ionizing radiations. *Int J Radiat Biol* 56: 623-634.
- Goodhead DT. 1994. Initial events in the cellular effects of ionizing radiation: clustered damage in DNA. *Int J Radiat Biol* 65: 7-17.
- Goodhead DT, Munson RJ, Thacker J, and Cox R. 1980. Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. IV. Biophysical interpretation. *Int J Radiat Biol Relat Stud Phys Chem Med* 37: 135- 167.
- Goodhead DT and Nikjoo H. 1997. Clustered damage in DNA: estimates from track- structure simulations. *Radiat Res* 148: 485-48.
- Goodhead DT, Thacker J, and Cox R. 1993. Weiss lecture. Effects of radiation of different qualities on cells: molecular mechanisms of damage and repair. *Int J Radiat Biol* 63: 543-556.
- Gulston M, Fulford J, Jenner T, de Lara C, and O'Neill P. 2002. Clustered DNA damage induced by gamma radiation in human fibroblasts (HF19), hamster (V79-4) cells and plasmid DNA is revealed as Fpg and Nth sensitive sites. *Nucleic Acids Res* 30: 3464-3472.
- Harrison L, Hatahet Z, Purmal AA, and Wallace S. 1998. Multiply damages sites in DNA: interactions with *Escherichia coli* endonucleases III and VIII. *Nucleic Acids Res* 26: 932-941.
- Harrison L, Hatahet Z, and Wallace S. 1999. In vitro repair of synthetic ionizing radiation-induced multiple damage DNA sites. *J Mol Biol* 290: 667-684.
- Hayes DP. 2006. DNA damage: risk comparisons of low radiation vis-à-vis dietary micronutrient deficiencies. *Int J Low Radiation* 3: 284-298.
- Hayes, DP. 2007. Nutritional hormesis. *Eur J Clin Nutr* 61: 147-159.
- Hayes DP. 2008. Aspects of the BEIR VII report: a critical evaluation. *Int J Low Radiation* 4 (no. 4): *in press*.
- Hooker AM, Bhat M, Day TK, Lane JM, Swinburne SJ, Morley AA., and Sykes PJ. 2004. The linear no-threshold model does not hold for low doses of ionizing radiation. *Radiat Res* 162: 447-452.
- Howard-Flanders P. 1958. Physical and chemical mechanisms in the injury of cells of ionizing radiations. *Adv Biol Med Phys* 6: 553-603.
- ICRP (International Commission on Radiological Protection). 1991. 1990 Recommendations of the International Commission on Radiological Protection, ICRP Publication 60, Annals of the ICRP, Vol 21. Pergamon Press, Oxford, pp 1- 3.
- ICRU (International Commission on Radiation Units and Measurements). 1983. Microdosimetry, ICRU-Report 36, Bethesda, MD, USA.

D. P. Hayes

- Ishizaki K, Hayashi Y, Nakamura H, Yasui Y, Komatsu K, and Tachibana A. 2004. No induction of p53 phosphorylation and few focus formation of phosphorylated H2AX suggest efficient repair of DNA damage during chronic low-dose-irradiation in human cells. *Radiat Res (Tokyo)* 45: 521-525.
- Joiner MC, Lambin P, Malaise EP, Robson T, Arrand JE, Skov KA, and Marples B. 1996. Hypersensitivity to very-low single radiation doses: its relationship to the adaptive response and induced radioresistance. *Mutat Res* 358: 171-183.
- Joiner MC, Marples B, Lambin P, Short SC, and Turesson I. 2001. Low-dose hypersensitivity: current status and possible mechanisms. *Int J Radiat Biol Phys* 49: 379-389.
- Ko M, Lao XY, Kapadia R, Elmore E, and Redpath JL. 2006. Neoplastic transformation *in vitro* by low doses of ionizing radiation: role of adaptive response and bystander effects. *Mutat Res* 597: 11-17.
- Koval TM. 1998. Enhanced recovery from ionizing radiation damage in a lepidopteran insect cell line. *Radiat Res* 115: 413-420.
- Krueger SA, Joiner MC, Weinfeld M, Piasentin E, and Marples B. 2007. Role of apoptosis in low-dose hyper-radiosensitivity. *Radiat Res* 167: 260-267.
- Lea DE. 1947. The structure of chromosome structural changes by radiation: detailed quantitative interpretations. *Br J Radiol, Supplement* 1: 75-83.
- Leonard BE. 2007. Thresholds and transitions for activation of cellular radioprotective mechanisms - correlations between HRS/IRR and the 'inverse' dose-rate effect. *Int J Radiat Biol* 83: 479-489.
- Löbrich M, Rief N, Kuhne M, Heckmann M, Fleckenstein J, Rube C, and Uder M. 2005. In vivo formation and repair of DNA double-strand breaks after computed tomography examination. *Proc Natl Acad Sci USA* 102: 8984-8989.
- Marples B. 2004. Is low-dose hyper-radiosensitivity a measure of G2-phase cell radiosensitivity? *Cancer Metastasis Rev* 23: 197-207.
- Marples B and Joiner MC. 2000. Modification of survival by DNA repair modifiers: a probable explanation for the phenomenon of increased radioresistance. *Int J Radiat Biol* 76: 305-312.
- Marples B, Wouters BG, Collis SJ, Chalmers AJ, and Joiner MC. 2004. Low-dose hyper-radiosensitivity: a consequence of ineffective cell cycle arrest of radiation-damaged G2-phase cells. *Radiat Res* 161: 247-255.
- Marples B, Wouters BG, and Joiner MC. 2003. An association between the radiation-induced arrest of G2-phase cells and low-dose hyper-radiosensitivity: a plausible underlying mechanism? *Radiat Res* 160: 38-45.
- Mercier G, Berthault N, Mary J, Peyre J, Antoniadis A, Comet JP, Cornuejols A, Froidevaux C, and Duteix M. 2004. Biological detection of low radiation doses by combining results of two microarray analysis methods. *Nucleic Acids Res* 32: e12.
- Milligan JR, Aguilera JA, Nguyen TT, Paglinawan RA, and Ward J. 2000. DNA strand-break yields after post-irradiation incubation with base excision repair endonucleases implicate hydroxyl radical pairs in double-strand break formation. *Int J Radiat Biol* 76: 1475-1483.
- Mitchell CR, Folkard M, and Joiner MC. 2002. Effects of exposure to low-dose-rate (60) Co gamma rays on human tumor cells in vitro. *Radiat Res* 158: 311-318.
- Mitchell CR and Joiner MC. 2002. Effects of subsequent acute-dose irradiation on cell survival in vitro following low dose-rate exposures. *Int J Radiation Biol* 78: 981-990.
- Mochan TA, Venere M, DiTullio RA Jr, and Halazonetis TD. 2003. 53PB1 and NFB1/MDC1-Nbs1 function in parallel interacting pathways activating ataxia-telangiectasia mutated (ATM) response to DNA damage. *Cancer Res* 63: 8586-8591.
- NCRP (National Conference on Radiation Protection and Measurement). 2001. Report No. 136. Evaluation of the Linear-Nonthreshold Dose-Response Model for Ionizing Radiation, Bethesda, Maryland.
- Nikjoo H and Goodhead DT. 1991. Track structure analysis illustrating the prominent role of low-energy electrons in radiobiological effects of low-LET radiations. *Phys Med Biol* 36: 229-238.
- Nikjoo H, O'Neill P, Terrissol M, and Goodhead DT. 1999. Quantitative modeling of DNA damage using Monte Carlo track structure method. *Radiat Environ Biophys* 38: 31-38.
- NRC (National Research Council of the National Academies). 2006. Health Risks from Exposure to Low Levels of Ionizing Radiation (BEIR VII, Phase 2). National Academies Press, Washington, DC.
- Pant MC, Liao XY, Lu Q, Molloy S, Elmore E, and Redpath JL. 2003. Mechanisms of suppression of neoplastic transformation in vitro by low doses of low LET radiation. *Carcinogenesis* 24: 1961-1965.

Nonproblematic risks from radiation damage clusters

- Pastwa E, Neumann RD, Mexhevaya K, and Winters TA. 2003. Repair of radiation-induced DNA double-strand breaks is dependent upon radiation quality and the structural complexity of double-strand breaks. *Radiat Res* 159: 251-261.
- Portess DI, Bauer G, Hill MA, and O'Neill P. 2007. Low-dose irradiation of nontransformed cells stimulates the selective removal of precancerous cells via intercellular induction of apoptosis. *Cancer Res* 67: 1246-1253.
- Prise KM, Pinto M, Newman HC, and Michael BD. 2001. A review of studies of ionizing radiation-induced double-strand break clustering. *Radiat Res* 156: 572-576.
- Prise KM, Pullar CH, and Michael BD. 1999. A study of endonuclease III-sensitive sites in irradiated DNA: detection of alpha-particle-induced oxidative damage. *Carcinogenesis* 20: 905-909.
- Radford IR. 2002. Model for the initiation of ionizing radiation-induced apoptosis in lymphoid cells by complex DNA double-strand breaks. *Int J Radiat Biol* 78: 467-474.
- Redpath JL. 2004. Radiation-induced neoplastic transformation *in vitro*: evidence for a protective effect at low doses of low LET radiation. *Cancer Metastasis Rev* 23: 333-339.
- Redpath JL. 2005. Nonlinear response for neoplastic transformation following low doses of low LET radiation. *Nonlinearity in Biology, Toxicology, and Medicine* 3: 113-124.
- Redpath JL. 2006a. Suppression of neoplastic transformation *in vitro* by low doses of low LET radiation. *Dose-Response* 4: 302-308.
- Redpath JL. 2006b. Health risks of low photon energy imaging. *Radiat Prot Dosimetry* 122: 528-533.
- Redpath JL and Elmore EE. 2007. Radiation-induced neoplastic transformation *in vitro*, hormesis and risk assessment. *Dose-Response* 5: 123-130.
- Redpath JL, Liang D, Taylor TH, Christie C, and Elmore E. 2001. The shape of the dose-response curve for radiation-induced neoplastic transformation *in vitro*: evidence for an adaptive response against neoplastic transformation at low doses of low-LET radiation. *Radiat Res* 156: 700-707.
- Redpath JL, Lu Q, Lao X, Molloy S, and Elmore E. 2003a. Low doses of diagnostic energy X-rays protect against neoplastic transformation *in vitro*. *Int J Radiat Biol* 79: 235-240.
- Redpath JL, Short SC, Woodcock M, and Johnston PJ. 2003b. Low-dose reduction in transformation frequency compared to unirradiated controls: the role of hypersensitivity to cell death. *Radiat Res* 59: 433-436.
- Rogakou EP, Pilch DR, Orr AH, Ivanova VS, and Bonner WM. 1998. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem* 273: 5858-5868.
- Rothkamm K and Löbrich M. 2003. Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses. *Proc Natl Acad Sci USA* 100: 5057-5062.
- Scott BR, Haque M, and Di Palma. 2007. Biological basis for radiation hormesis in mammalian cellular communities. *Int J Low Radiation* 4: 1-16.
- Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lobrich M, and Jeggo PA. 2004. ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. *Cancer Res* 64: 2390-2396.
- Sutherland BM, Bennett PV, Schenk H, Sidorkina O, Laval J, Trunk J, Monteleone D, and Sutherland J. 2001. Clustered DNA damages induced by high and low LET radiation, including heavy ions. *Phys Med* 17 Suppl 1: 202-204.
- Sutherland BM, Bennett PV, Sidorkina O, and Laval J. 2000. Clustered DNA damages induced in isolated DNA and in human cells by low doses of ionizing radiation. *Proc Natl Acad Sci USA* 97: 103-108.
- Sutherland BM, Bennett PV, Sutherland JC, and Laval J. 2002. Clustered DNA damages induced by x rays in human cells. *Radiat Res* 157: 611-616.
- Sutherland BM, Georgakilas AG, Bennett PV, Laval J, and Sutherland JC. 2003. Quantifying clustered DNA damage induction and repair by gel electrophoresis, electron imaging and number average length analysis. *Mut Res* 53: 93-107.
- Sykes PJ, Day TK, Swinburne SJ, Lane JM, Morley AA, and Hooker AM. 2006a. In vivo mutagenic effect of very low dose radiation. *Dose-Response* 4: 309-316.
- Sykes PJ, Morley AA, and Hooker AM. 2006b. The pKZ1 recombination mutation assay: a sensitive assay for low dose studies. *Dose-Response* 4: 91-105.
- Tubiana M, Aurengo A, Auerbeck D, Bonnin A, LeGuen B, Masse R, Monier R, Valleron AJ, and de Vathaire F. 2005. Dose-effect relationships and estimation of the carcinogenic effect of low doses of ionizing radiation, Académie des Sciences - Académie Nationale de Médecine Report, Paris, France.

D. P. Hayes

- Tubiana M, Aurengo A, Auerbeck D, and Masse. 2006. Recent reports on the effect of low doses of ionizing radiation and its dose-effect relationship. *Radiat Environ Biophys* 44: 245-251.
- United Nations. 2000. UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) Sources and Effects of Ionizing Radiation, Report to the General Assembly with Scientific Annexes, Volume II: Effects. New York.
- Vilenchik MM and Knudson AG Jr. 2000. Inverse radiation dose-rate effects on somatic and germ-line mutations and DNA damage rates. *Proc Natl Acad Sci USA* 97: 5381-5386.
- Vilenchik MM and Knudson AG Jr. 2006. Radiation dose-rate effects, endogenous DNA damage, and signaling resonance. *Proc Natl Acad Sci USA* 103: 17874-17879.
- Wallace SS. 1998. Enzymatic processing of radiation-induced free radical damage in DNA. *Radiat Res* 150: S60-S79.
- Ward JF. 1981. Some biological consequences of the spatial distribution of ionizing radiation-produced free radicals. *Radiat Res* 86: 185-195.
- Ward JF. 1985. Biochemistry of DNA lesions. *Radiat Res Suppl* 104: S103-S111.
- Ward JF. 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 JF. 1991. Mechanisms of radiation action on DNA in model systems - their relevance to cellular DNA. In: Felden EM and O'Neill P (eds.), *The Early Effects of Radiation on DNA*, pp 1-16, Springer-Verlag, Berlin Heidelberg.
- Ward JF. 1994. The complexity of DNA damage: relevance to biological consequences. *Int J Radiat Biol* 66: 427-432.
- Ward JF. 2000. Complexity of damage produced by ionizing radiation. *Cold Spring Harb Symp Quant Biol* 65: 377-382.
- Yang N, Chaudhry MA, and Wallace SS. 2006. Base excision repair by hNTH1 and hOGG1: a two edged sword in the processing of DNA damage in gamma- irradiated human cells. *DNA Repair (Amst)* 5: 43-51.
- Yang N, Galick H, and Wallace SS. 2004. Attempted base excision repair of ionizing radiation damage in human lymphoblastoid cells produce lethal and mutagenic double strand breaks. *DNA Repair (Amst)* 3: 1323-1334.
- Yin E, Nelson DO, Coleman MA, Peterson LE, and Wyrobek AJ. 2003. Gene comparison changes in mouse brain after exposure to low-dose ionizing radiation. *Int J Radiat Biol* 79: 759-775.
- Zeng G, Day TK, Hooker AM, Blyth BJ, Bhat M, Tilley, W.D., and Sykes PJ. 2006. Non-linear chromosomal inversion response in prostate after low dose X-radiation exposure. *Mutat Res* 602: 65-73.