

COMMENTARY

Spontaneous DNA Damage and Its Significance for the "Negligible Dose" Controversy in Radiation Protection

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One of the crucial problems in radiation protection is the reality of the negligible dose or *de minimus* concept (1-4). This issue of a "practical zero" and its resolution is central to our understanding of the controversy concerning the existence of a "safe" dose in radiological health. However, for very low levels of environmental mutagens and carcinogens including low doses of low-LET radiations (less than 1 cGy or 1 rad), spontaneous or endogenous DNA damage may have an increasing impact on the biological consequences of the induced cellular response. It is this issue that is addressed in this communication.

The following discussion is intentionally limited to a comparison of low-LET radiation since its effects are due primarily to indirect damage in cellular DNA brought about by OH radicals. Indirect effects of low-LET radiation under aerobic conditions are reported to account for 50-85% of measured radiation damage in cells (5, 6). High-LET radiation, on the other hand, produces unique DNA damage (7) primarily by direct effects (5) which is less likely to be properly repaired (7).

Spontaneous or intrinsic modification of cellular DNA is ubiquitous in nature and likely to be a major cause of background mutations (8), cancer (9), and other diseases (10). The documentation of this intrinsic DNA decay has increased at a rapid pace in recent years and has not gone unnoticed by contemporary radiobiologists. Setlow (11) and more recently Saul and Ames (12) summarized the findings of Lindahl and Karlstrom (13) and others (14) which suggest that approximately 10,000 measurable DNA

modification events occur per hour in each mammalian cell due to intrinsic causes.

The current radiation literature will be interpreted to show that ~100 (or fewer) measurable DNA alterations occur per centigray of low-LET radiation per mammalian cell. Therefore every *hour* human and other mammalian cells undergo at least 50-100 times as much spontaneous or natural DNA damage as would result from exposure to 1 cGy of ionizing radiation. Since background radiation is usually less than 100-200 mrem (1-2 mSv)/y, it can be concluded, as discussed by Muller and Mott-Smith (15), that spontaneous DNA damage is due primarily to causes other than background radiation.

"INTRINSIC" OR "SPONTANEOUS" DNA DAMAGE

DNA is not as structurally stable as once thought. On the contrary, there appears to be a natural background of chemical and physical lesions introduced into cellular DNA by thermal as well as oxidative insult. In addition, in the course of evolution, many cells have evolved biochemical mechanisms for repair or bypass of these lesions.

Some of the more common "natural" DNA changes include depurination, depyrimidination, deamination, single-strand breaks (SSBs), double-strand breaks (DSBs), base modification, and protein-DNA crosslinks. These are caused by thermodynamic decay processes as well as reactive molecules formed by metabolic processes leading to free radicals such as OH, peroxides, and reactive oxygen species.

Shapiro (14) has recently discussed and summarized the frequency at which various kinds of spontaneous DNA damage occur. Spontaneous DNA damage events per cell per hour are shown in Table I and were estimated from the data presented by Shapiro [Table II (14)].

For single-stranded DNA of mammalian cells at least 8×10^3 damage events occur/cell/h, whereas for double-stranded DNA there were $\sim 6 \times 10^3$ damage events per hour (Table I). While the ratio of single-stranded DNA to

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TABLE I
Estimated Spontaneous DNA Degradation Events (Cell/h)^a

Reaction	Single-strand DNA	Double-strand DNA
Depurination	4000	1000
Depyrimidination	200	50
Deamination of cytosine	4000	15
Chain break resulting from depurination	—	1000
Direct chain break	—	4000

^a Calculated from Shapiro (14).

double-stranded DNA varies with phase of the cell cycle, it is reasonable to assume that double-stranded DNA is the usual configuration for most cellular DNA at any one time. From the data summarized in Table I it is not unreasonable to suggest that, at a minimum, the spontaneous DNA damage is of the order of $6-10 \times 10^3$ events/cell/h and to use 8×10^3 DNA damage events/cell/h as a reasonable average for the purpose of discussion. This allows a calculation of 1.9×10^5 spontaneous cellular DNA damaging events/cell/day or 7×10^7 per year in mammals including humans (Table II). The lifetime load of spontaneous DNA damage events per cell is then $\sim 5 \times 10^9$ if an average life span of 75 years is allowed for humans.

DNA DAMAGE INDUCED BY IRRADIATION

Several recent reviews summarize the types and quantities of alteration of DNA in cells caused by exposure to low-LET radiation (16–18). The reader should refer to these for references to the original works from which the reviews were drawn.

The estimate of about 100 DNA events/cell/cGy used in this discussion is based on information contained in the

reviews by Ward (16, 20) and assumes the molecular weight of the mammalian genomic DNA to be 6×10^{12} Da, constituting about 1% of the cell weight.

Ward [Table II (16)] lists the amount of energy deposited in various DNA constituents/cell/Gy. From this table a total of 13.3 DNA events/cGy is calculated. His estimate of damaged DNA sites/cell/cGy is 10–100. I chose the 100-lesion estimate to make as reasonable a conservative comparison with spontaneous DNA damage as possible (Table II). This number of damaged sites would include both direct and indirect DNA damage.

SPONTANEOUS VS INDUCED DNA MODIFICATIONS AND THEIR BIOLOGICAL CONSEQUENCES

Wallace has recently reviewed the nature of the DNA lesions caused by active oxidizing species produced both naturally and by low-LET radiation (17). Oxidizing radicals and especially OH radicals resulting from either cause produce similar types of DNA lesions (17–19). The enzymes involved in their repair are similar whether the DNA damage is produced spontaneously or by radiation. However, radiation is known to induce an error-prone repair system in bacterial cells and perhaps in mammalian cells as well (21, 22).

DNA glycosylases and endonucleases are involved in the repair of base damage. Other nucleases are available for sugar damage repair (17). Recognition of the damage site by the appropriate enzymes is dependent not on the initiating event but on the chemical nature of the end product. These end products appear to be similar whether induced by natural causes or radiation (17). It would seem reasonable to conclude that, due to common oxidizing radicals, many of the qualitative changes in DNA are quite similar for radiation-induced or spontaneous DNA damage.

TABLE II
DNA Damage Events per Mammalian Cell

Character of event	Spontaneous DNA damage events			DNA damage/cGy ^a
	Per second	Per hour	Per year	
Single-strand breaks	1.4	$\sim 5 \times 10^3$	$\sim 4.4 \times 10^7$	10
Double-strand breaks				0.4
Depurination and/or base lesions	0.8	$\sim 1.5 \times 10^3$ $\sim 1.25 \times 10^3$	$\sim 1.4 \times 10^7$ $\sim 1.1 \times 10^7$	9.5
Total events	2.2	$\sim 8.0 \times 10^3$	$\sim 7 \times 10^7$	~ 20
cGy equivalents (1 cGy = 100 events) ^b	0.022	8.0×10^1	7×10^5	

^a From Ward (20).

^b Since other radiation-induced DNA damage such as DNA–protein crosslinking and base modifications (18) occur, 100 events/cGy is used as a “ballpark” value for ease of comparison with spontaneous events.

The quantity and distribution of each class of lesion may, however, differ significantly. As indicated earlier there would appear to be relatively more DNA strand breaks than other lesions resulting from spontaneous causes as compared to radiation insult. A good portion of these may result from depurination (Table I) with production of 3' OH termini ("clean ends") as part of the repair process.

Many of the DNA strand breaks caused by low-LET radiation are incapable of serving as primer for DNA polymerase (23). However, endo- and exonucleases exist which can restore these blocking ends to clean ends and allow completion of the repair process (17).

A strong correlation exists between DNA DSBs and lethality in mammalian cells for low-LET radiation. While the quantity of DSBs produced by ionizing radiation is fairly well documented, this is not true for spontaneous DSB production in mammalian cells.

In spontaneous DNA decay, formation of a DSB is likely to be the result of single-strand events occurring in close proximity on each daughter strand and leading to cohesive ends which can be repaired easily by a ligation step.

A survey of the literature on the doubling dose for mutagenesis in eukaryotes exposed to low-LET radiation indicates a range of 4 to 300 cGy and for carcinogenesis a range of 100 to 400 cGy. Using the "ballpark" value of approximately 100 DNA events/cell/cGy, this would represent a range of 400 to 40,000 induced DNA damage events per doubling dose. Using 100 cGy as the approximate doubling dose, a total of 1×10^4 DNA damage events would be required to induce mutations in numbers equal to that observed in nature. This is approximately the number of DNA events (8.0×10^3) produced spontaneously in each cell/h (Table II).

THE NEGLIGIBLE DOSE CONTROVERSY

The comparison of low-LET radiation-induced DNA damage with that which occurs spontaneously indicates (Table II) that a relatively large number of DNA damage events can occur spontaneously during the lifetime of mammalian and other cells.

Dose protraction over a period of weeks or months would lead to an increasing ratio of spontaneous DNA damage events to those caused by irradiation. By extrapolation from high doses and high dose rate as discussed by Ward (16, 20), 1 cGy delivered in 1 s would cause 40–50 times as many DNA damaging events per cell as that caused spontaneously during the same time span (Table II). However, 1 cGy delivered evenly over 1 year would cause (on average) less than 1 DNA damaging event per cell/day. This can be compared to $\sim 2 \times 10^5$ natural events caused per cell/day.

From these numbers, it seems reasonable to suggest that there does exist a "negligible" dose in the range of our terrestrial background annual radiation dose of ~ 1 mSv (~ 10

DNA events/cell/year). This can be compared to the approximately 7×10^7 DNA events/cell/years produced by spontaneous causes.

Adler and Weinberg (24) have proposed that the standard deviation of the background irradiation (~ 0.2 mSv) be used as an acceptable additional dose due to human activities. This would lead to ~ 2 additional induced DNA damaging events/cell/year as compared to $\sim 7 \times 10^7$ spontaneous DNA damage events. Considering the magnitude of the spontaneously induced DNA changes in each human cell, it is not unreasonable to predict that 0.2 mSv delivered over a year would have negligible biological consequences.

When temporal considerations are factored in, it becomes clear that spontaneous DNA damage in mammalian cells may be many orders of magnitude greater than that caused by low and protracted radiation doses, especially in the terrestrial background range of 1–2 mSv (100–200 mrem) per year. It is important that further studies on the effects of both ionizing radiations and spontaneous events on DNA decay and repair be conducted to better understand the practical health consequences of low and protracted doses of radiation (2, 9, 25).

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