R.D. Stewart, On the Complexity of the DNA Damages Created by Endogenous Processes, *Radiat. Res.*, **152**(1), 101-105 (1999).

In an earlier commentary, Billen suggested that the biological effects of ionizing radiation in the low dose and dose-rate regime of interest in radiation protection may be negligible when contrasted with endogenous or "spontaneous" DNA damaging events (*1*). In a response to Billen, Ward challenged this suggestion on the basis that the biological effects of equal *quantities* of radiation- and endogenously created DNA damage are not the same because endogenous processes cannot easily create "Locally Multiply Damaged Sites" (LMDS) and in particular double strand break (DSB) lesions (*2*).

Although I agree with Ward that the quality of the DNA damage is an important factor that must be considered in addition to the quantity of damage, the arguments of Billen and Ward may have been too simplistic in some respects. Calculations are presented below that suggest endogenous processes may be capable of producing at least one type of LMDS – simple DSB lesions – at a rate comparable to an annual radiation dose on the order of about 950 mGy. It also seems likely that endogenous processes are capable forming other types of relatively simple LMDS at levels equivalent to a 950 mGy annual dose. Although these calculations do not negate all of Ward's arguments against the existence of a "negligible dose," it does suggest that simple double strand break lesions and other classes of simple LMDS can be formed by endogenous processes and are not unique to ionizing radiation.

RATE OF ELEMENTARY DAMAGE SITE FORMATION IN A MAMMALIAN CELL

Before considering the rate of double strand break formation by endogenous processes, it is useful to introduce some terminology and briefly review the rate endogenous processes create simple DNA damages. For the purposes of characterizing different types of DNA damage, two types of "elementary damage sites" (EDS) are suggested: base alterations (Ba) and strand breaks (Sb). A base alteration occurs when the chemical properties of an organic base (A, T, G, or C) are abnormally modified from

the form found in undamaged DNA. An example of a base alteration is the deamination of cytosine to form uracil. In this work, the loss of purines and pyrimidines from the DNA (a "base deletion") are included in the base alteration category of EDS. A strand break occurs when the covalent bond between the deoxyribose sugar unit and the phosphate group (bridge) is severed. By definition, the nucleotide on the 5′ side of the severed sugar-phosphate bond is arbitrarily designated as the site of the elementary damage site.

Friedberg *et al.* (*3*) used the method of Shapiro (*4*) to estimate the frequency of spontaneous base altering events in double-stranded DNA on an hourly basis. From these data, the number of spontaneous base alterations can be estimated at 1.1×10^{-7} Ba h^{-1} per base pair (bp). For a typical mammalian cell with a DNA content of 6.6×10^{9} bp, the number of base alterations per hour is thus estimated at about 700 Ba h^{-1} $cell^{-1}$. In contrast, Billen estimated the number of base altering events per hour in double-stranded DNA at about 1065 Ba h^{-1} $cell^{-1}$ (*1*). The rate of endogenous strand break formation in a typically mammalian cell is on the order of about 5000 Sb h^{-1} $cell^{-1}$ (*1*).

RATE OF DOUBLE STRAND BREAK PRODUCTION BY ENDOGENOUS PROCESSES

Suppose that a DNA lesion is defined as any 40 base pair segment of the DNA that contains one or more elementary damages sites. Suppose further that endogenously created elementary damage sites are formed at random locations in the DNA. Then, it follows that, *in undamaged regions of the DNA*, endogenous processes can only create two types of DNA lesions: lesions composed of a single base alteration (a Ba lesion) and lesions composed of one strand break (a Sb lesion or a "single strand break"). Although excision repair mechanisms are capable of rapidly repairing simple lesions such as these, for the brief period of time in which these lesions are present in the DNA, the creation of a second elementary damage site near an existing lesions will transform this lesion into a more complex type of damage, e.g., a double strand break (DSB) or a strand break with a nearby base alteration. A method of estimating the rate single strand breaks are transformed into double strand breaks by endogenous processes is described next.

Let $\overline{L}_{Sb}(t)$ be the expected number of Sb lesions (single strand breaks) formed in a cell at time *t*. The net time-rate of change in $\overline{L}_{Sb}(t)$ equals the rate new strand breaks are formed in undamaged regions of the DNA minus the expected rate Sb lesions are (correctly or incorrectly) repaired minus the rate Sb lesions are transformed into a more complex type of damage by the creation of another EDS near an existing lesion. Let P_{Sb} be the expected number of Sb lesions produced in a cell per unit time, let P_{Ba} denote the expected number of Ba lesions produced in a cell per unit time, and let $\gamma_{Sb\to j}(k)$ be the probability, per EDS, that a Sb lesion is transformed into a *j*th type of lesion when a *k*th type of elementary damage site is created in the DNA.

The net time-rate of change in $\overline{L}_{Sb}(t)$ can now be expressed mathematically as

$$\begin{aligned}\frac{d\overline{L}_{Sb}(t)}{dt} &= P_{Sb} - \lambda_{Sb}\overline{L}_{Sb}(t) - \overline{L}_{Sb}(t)\sum_{k}\sum_{j} P_k \cdot \gamma_{Sb\to j}(k) \\ &= P_{Sb} - \overline{L}_{Sb}(t)\left\{\lambda_{Sb} + \overline{\gamma}_{Sb}\left(P_{Sb} + P_{Ba}\right)\right\},\end{aligned} \tag{1}$$

where λ_{Sb} is the probability that a Sb lesion is repaired correctly or incorrectly per infinitesimal unit of time (a "lesion repair probability") and $\overline{\gamma}_{Sb}$ is the probability, per EDS, that a Sb lesion is transformed into some other type of lesion by the creation of an additional elementary damage site in the DNA. Because the creation of a new EDS near an existing Sb lesion will, by definition, transform a simple Sb lesion into a different (more complex) type of damage, $\overline{\gamma}_{Sb}$ must simply equal the probability, per EDS, that a randomly formed elementary damage site will be created close (about 20 base pairs) to an existing Sb lesion.

Suppose at time $t = 0$, the initial number of Sb lesions in the cell is zero so that $\overline{L}_{Sb}(0) = 0$. After rearranging terms, Eq. (1) can be integrated analytically to obtain, for $t > 0$,

$$\overline{L}_{Sb}(t) = \frac{P_{Sb}}{\lambda_{Sb} + \overline{\gamma}_{Sb}\left(P_{Sb} + P_{Ba}\right)}\left\{1 - \exp-\left[\lambda_{Sb} + \overline{\gamma}_{Sb}\left(P_{Sb} + P_{Ba}\right)\right]t\right\}. \tag{2}$$

In the limit as $t \to \infty$ (i.e., at equilibrium), Eq. (2) simplifies to

$$\overline{L}_{Sb}(t_\infty) \equiv \overline{L}_{Sb}^{\infty} = \frac{P_{Sb}}{\lambda_{Sb} + \overline{\gamma}_{Sb}\left(P_{Sb} + P_{Ba}\right)}. \tag{3}$$

A double strand break is formed when two strand breaks are formed on opposite sides of the DNA within about 20 base pairs (*5*). Under equilibrium conditions, the rate double strand breaks are created by endogenous processes is the product of the transformation probability $\gamma_{Sb\to dsb}$, the rate of new Sb formation P_{sb}, and $\overline{L}_{Sb}^{\infty}$. Thus,

$$P_{dsb} = \gamma_{Sb\to dsb} \cdot P_{Sb} \cdot \overline{L}_{Sb}^{\infty} = \frac{\gamma_{Sb\to dsb}\left(P_{Sb}\right)^2}{\lambda_{Sb} + \overline{\gamma}_{Sb}\left(P_{Sb} + P_{Ba}\right)}. \tag{4}$$

Suppose that the DNA content of a cell is *N* base pairs. Under the assumption that strand breaks are formed at random locations in the DNA by endogenous processes, it follows that the Sb transformation probability $\overline{\gamma}_{Sb}$ is approximately equal to $\overline{\gamma}_{Sb} = 40 \text{ bp}/2\text{N} = 20\text{N}^{-1}$. The strand break to double strand break transformation probability $\gamma_{Sb\to dsb}$ is equal to the probability a new strand break is formed near an existing Sb lesion times the probability this strand break occurs in the DNA strand opposite to the existing strand break. Since $\overline{\gamma}_{Sb} \cdot P_{Sb}/(P_{Sb} + P_{Ba})$ is the probability, per EDS, that a new strand Sb will be created near an existing Sb lesion, $\gamma_{Sb\to dsb}$ is equal to

$$\gamma_{Sb\to dsb} = \frac{1}{2}\frac{\overline{\gamma}_{Sb} \cdot P_{Sb}}{\left(P_{Sb} + P_{Ba}\right)} = \frac{10P_{Sb}}{\left(P_{Sb} + P_{Ba}\right)N} \tag{5}$$

Thus, Eq. (4) becomes

$$P_{dsb} = \gamma_{Sb\to dsb}\left(P_{Sb}\right)\overline{L}_{Sb}^{\infty} = \frac{10\left(P_{Sb}\right)^3}{\lambda_{Sb}\left(P_{Sb} + P_{Ba}\right)N + 20\left(P_{Sb} + P_{Ba}\right)^2}. \tag{6}$$

For simple lesions such as individual base damages and strand breaks, repair mechanisms are available to rapidly and accurately repair the damage. For single strand breaks, the rate of damage repair most likely corresponds to a repair probability λ_{Sb} in the range from about 2.77 h^{-1} (15 minute repair half-time) to 16.6 h^{-1} (2.5 minute repair

half-time) (*6*). From the previous section, it seems likely that P_{Sb} is most likely in the range from 2500 to 7500 Sb h^{-1} $cell^{-1}$, and P_{Ba} is most likely less than about 1400 Ba h^{-1} $cell^{-1}$. For a typically mammalian cell with a DNA content of 6.6×10^9 bp, $\overline{\gamma}_{Sb}$ is on the order of about 3.0×10^{-9} and $\gamma_{Sb \to dsb} \cong 1.3 \times 10^{-9}$.

Although all of the parameters in Eq. (6) can be constrained to a meaningful range of values, the possible range of parameters values is such that the estimated rate of double strand break production, P_{dsb}, can vary by almost two orders of magnitude depending on the particular values selected for λ_{Sb}, P_{Ba}, P_{Sb}, and N. Because it is difficult to judge which set of values are the best ones, Monte Carlo techniques have been used to select random parameter values from the phase space $2.77\ h^{-1} < \lambda_{Sb} < 16.6\ h^{-1}$, $0 < P_{Ba} < 1400$ Ba $cell^{-1}$ h^{-1}, and $6.0 \times 10^9 < N < 7.2 \times 10^9$. For a specified value of P_{Sb}, the double strand break production rate was then computed using Eq. (6) and recorded.

After a larger number of random values for P_{dsb} were generated and recorded, a probability density function was constructed to describe the relative frequency of possible DSB formation rates. This probability density function was then used to compute a sample-mean DSB formation rate and a confidence interval that contains 95% of all of the randomly sampled DSB formation rates. The results of this Monte Carlo simulation are summarized in Figure 1. The rate of double strand break formation by endogenous processes in Figure 1 is converted to an equivalent low LET dose rate by multiplying P_{dsb} by 2.1915×10^5 (mGy y^{-1})/(DSB $cell^{-1}$ h^{-1}); this conversion factor corresponds to a typical double strand break yield of 40 DSB Gy^{-1} $cell^{-1}$ [for a review of DSB yields, see reference (*7*)].

Because of the uncertainties associated with the determination of input parameters for Eq. (6), it seems reasonable to use the sample-mean DSB formation rate at $P_{Sb} = 5000$ Sb h^{-1} $cell^{-1}$ as the "best estimate" of the rate of DSB formation by endogenous processes. The best estimate of the DSB formation rate for this value of P_{Sb} is 4.33×10^{-3} DSB $cell^{-1}$ h^{-1}. In terms of low-LET radiation, this DSB formation rate is equivalent to a dose rate of

about 950 mGy y^{-1}. Regardless of the accuracy of this best estimate value for P_{dsb}, it seems very likely that the true DSB formation rate by endogenous processes lies somewhere in the range from 90 to 5500 mGy y^{-1} – several orders of magnitude larger than background radiation (*8*).

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REFERENCES

1. D. Billen, "Commentary: Spontaneous DNA Damage and its Significance for the 'Negligible Dose' Controversy in Radiation Protection," *Radiat. Res.*, **124,** p. 242-245 (1990).

2. J.F. Ward, "Response to Commentary by D. Billen," *Radiat. Res.*, **126**, p. 385-387 (1991).

3. E.C. Friedberg, G.C. Walker, and W. Siede, Table 1-3, p. 9 *In* "DNA Repair and Mutagenesis," ASM Press, Washington, D.C. (1995).

4. R. Shapiro, "Damage to DNA caused by hydrolysis," p. 3-12 *In* E. Seeberg and K. Kleppe (ed.), *Chromosome Damage and Repair*. Plenum Publishing Corp., New York (1981).

5. F. Mingot, J.L. Jorcano, and C.A. Davila, "Local Perturbation of the Double Helical DNA Structure Near Single Breaks," *Biochimica et Biophysica Acta*, **520**, p. 1-10 (1978).

6. M. Frankenberg-Schwager, Induction, repair and biological relevance of radiation induced DNA lesions in eukaryotic cells, *Radiat. Environ. Biophys.*, **29**, 273-292 (1990).

7. V.V. Moiseenko, R.N. Hamm, A.J. Waker, and W.V. Prestwich, Table 6 in "Modelling DNA damage induced by different energy photons and tritium beta-particles," *Int. J. Radiat. Biol.*, **74**(5), p. 533-550 (1998).

8. NCRP, *Exposure of the Population in the United States and Canada from Natural Background Radiation*, Report 94, National Council on Radiation Protection and Measurements, Washington, D.C., (1987).

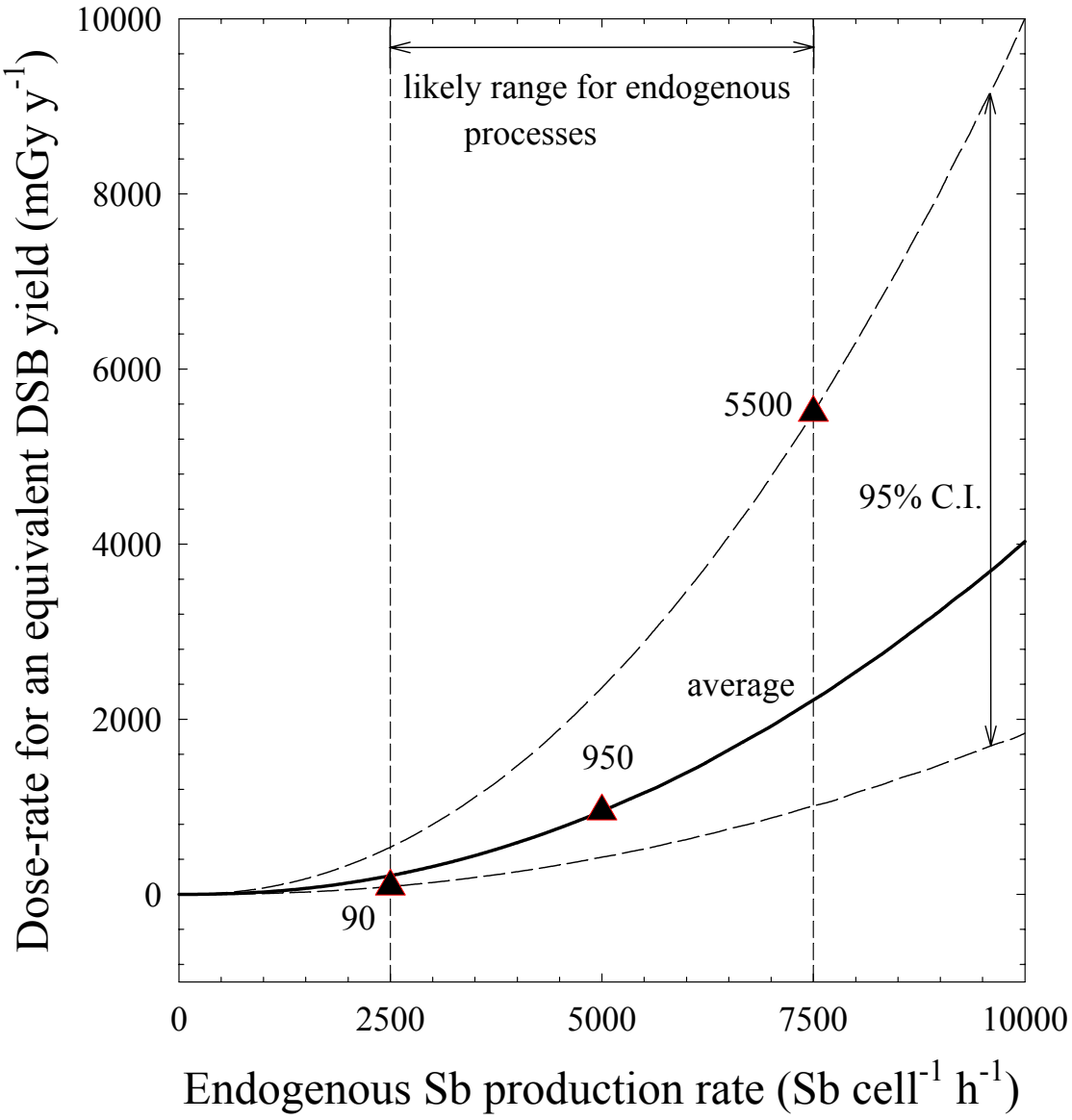

Figure 1. The rate of double strand break formation by endogenous processes. The DSB formation rate P_{dsb} in units of DSB cell^{-1} h^{-1} is multiplied by 2.1915×10^{5} (mGy y^{-1})/(DSB cell^{-1} h^{-1}) to convert the formation rate by endogenous processes to an equivalent low LET dose rate.