

Limits imposed by ionizing radiation on the long-term survival of trapped bacterial spores: beta radiation

A. J. NICASTRO^{†*}, R. H. VREELAND[‡] and W. D. ROSENZWEIG[‡]

(Received 9 January 2002; accepted 10 May 2002)

Abstract.

Purpose: A model is presented for determining the survival time T_F of a fraction F of a population of bacterial spores trapped within a fluid inclusion and subject to genetic damage from beta radiation.

Methods: The limiting factor to survival is the production of double-strand breaks (DSB) in the DNA resulting from single-track cleaving and from the cumulative effects of single-strand breaks (SSB) induced by the presence of ionizing radiation in the environment. The model considers the probability that radicals and ions formed by the passage of high-energy particles will interact with a DNA molecule and induce damage.

Results: The survival time T_F for a fraction F of a trapped population is a weak function of both F and the length L in base pairs of the genome. For irradiation due to a beta source trapped with the spores within the inclusion, the survival time is also inversely proportional to the concentration of the radionuclide, the dominant factor in limiting survival time.

Conclusions: The predictions of the model are consistent with measured DSB formation rates, the observed survival of trapped spores over time periods as long as 250 Ma, and track structure models which address low physical dose rates.

1. Introduction

Some species and strains of bacteria (i.e. *Bacillus* and *Clostridium*) are able, when conditions are adverse, to enter a dormant spore state wherein metabolic activity functionally ceases. Such spores, trapped within crystal inclusions, may lie dormant for extended time periods, protected to some degree by the encasing crystal. Vreeland *et al.* (2000) revived a *Bacillus* species (strain 2-9-3) trapped in a halite fluid inclusion 600 m below the surface for 250 Ma (250 million years). Parkes (2000) comments that, given the care taken to avoid contamination, that study presents the best evidence for the extreme longevity possible in micro-organisms. A question naturally arises as to what limits the long-term survival of trapped bacteria. In this paper, the limits imposed by the presence of beta radiation on the survival of

bacterial spores trapped within fluid inclusions of crystals is examined.

Bacterial spores trapped within a fluid inclusion are subject to exposure to ionizing radiation. This radiation may originate from radionuclides within the fluid, in the encasing crystal, or from crustal rock surrounding the crystal. This paper shows that the flux of alphas and of high-energy particles comprising cosmic rays is a negligible factor compared to the beta dose. The fact that Vreeland *et al.* (2000) were able to revive 250 Ma bacilli indicates that these organisms are rather robust, and that, at least in that case, the exposure was insufficient to kill all the trapped spores.

Lesions on a DNA molecule may be created by the near-passage of a high-energy particle such as might be emitted in radioactive decay. A direct collision of the particle with a constituent atom of the DNA may sever structural bonds by dislodging or ionizing the atom, or the high-energy particle can also create highly reactive radicals in the vicinity of a DNA strand which can subsequently attack critical sites on the molecule and sever structural bonds. The electric field of a relativistic particle is oriented so that the greatest field strength lies perpendicular to the particle's velocity (Jackson 1975). Passage of such a particle through a fluid produces ions several nanometers on either side of its trajectory (Turner 1995). If the fluid through which the particle moves has water as its dominant constituent, such as would be expected to be the case for bacterial DNA, ionizing radiation generally gives rise to H_2O^+ , H_3O^+ , OH , OH^- and some H_2O_2 and H_2 . Some of these products are the result of radicals interacting with themselves (Ward 1988). Along with direct ionization, the action of these radicals formed by water radiolysis produces lesions and deformations in DNA.

2. Lethal damage to DNA

The most significant types of damage to DNA that can lead to cell death are recognized to be double-strand breaks (DSB) and the accumulation of

*Author for correspondence. e-mail: anicastro@wcupa.edu

[†]Department of Physics, West Chester University of Pennsylvania, West Chester, PA 19383, USA.

[‡]Department of Biology, West Chester University of Pennsylvania, West Chester, PA 19383, USA.

single-strand breaks (SSB). One SSB is not problematic for an active cell. Efficient, accurate repair of the damage can be implemented so long as the integrity of the undamaged complementary strand is not compromised. No loss of base-sequence information occurs. If two SSB occur on opposite strands of a molecule, they can still be repaired as if they were singly damaged sites if they are separated by a sufficient number of base pairs. Two individual SSB may occur in opposite strands of a DNA molecule close enough so that any base pairs remaining between the damage sites do not provide enough structural binding to hold the strands together. A DSB forms, and the DNA molecule effectively is cut into smaller fragments. This lesion results in a loss of base sequence information and can result in cell death (Freifelder 1965, Frankenberg *et al.* 1981, Barendsen 1990, Frankenberg-Schwager and Frankenberg 1990, Sachs *et al.* 1997, Olive 1998). The trajectory of a high-energy particle may be oriented so that it lies along a line formed by a base pair. In such a circumstance, the particle may cleave the DNA and form a single-track DSB.

Mingot *et al.* (1978) analyzed the local deformations in a DNA double helix in the vicinity of a strand break. They found that two SSB on opposite strands within about 20 base pairs permits the complementary strands to denature and thus separate completely. In a comprehensive analysis, Freifelder and Trumbo (1969) showed that the number of base pairs that can retain the structural integrity of the DNA molecule varies with the ionic strength of the medium in which the DNA finds itself. They found that for media of high ionic strength (1.0 M NaCl), as few as 2.64 base pairs, on average, can prevent a DSB from forming. Note that a high concentration of salt ions (a saturated salt brine is 6 M) also act as scavengers for the radicals created by the passage of a high-energy charged particle, and thus serve to reduce the damage induced in the DNA. This aspect will be quantified in a later section of this paper.

Although in the present analysis the formation of a DSB is considered to be a lethal event, it is noted that it has been demonstrated that not all DSB are lethal. The initial number of DSB created by exposure to ionizing radiation often is 40–100 times higher than the frequency of lethal events (Ward 1988, see also Ward 1990). Further, at least two independent mechanisms (homologous recombination and non-homologous end-joining) permit the repair of DSB in prokaryotes and eukaryotes (Glasunov *et al.* 1989, Camerini-Otero and Hsieh 1995, Frieberg *et al.* 1995, Ivanov *et al.* 1996, Shinagawa 1996, Eisen and Hanawait 1999). Although cells are evidently capable of repairing

many DSB, Frankenberg *et al.* (1981) found about one unrejoined DSB per cell per lethal event. While dormant, the metabolic repair pathways are inactive or disabled. Thus, a spore exposed to a sufficient number of attacks from ionizing radiation may emerge from the dormant state with extensive damage to its genome, and DSB repair may be significantly less effective than when the cell is active while suffering DNA lesions. If the DNA of a spore suffers a DSB, the chemically reactive terminal ends of the strands at the breaks are free to diffuse and interact with nearby molecules or other regions of the DNA. Thus, when one of these chemically reactive ends diffuses away from its proper site on the helix before it can be repaired, the DSB damage may be irreparable. In this analysis, the production of a single DSB in a DNA molecule will be considered to be a fatal lesion leading ultimately to cell death. Regardless of the efficiency with which DSB can be repaired after dormancy is broken, the formation of fatal lesions will be modeled as the cumulative effects of non-repaired SSB coupled with single-track DSB. As a consequence of this approach, all that is required to predict the probability of survival for a population of trapped spores is a model to estimate the occurrence and distribution of fatal lesions produced by ionizing radiation. In the authors' model, it is assumed that all spores with one or more fatal lesions in their DNA die; the others survive.

Damage to DNA produced by ionizing radiation can be viewed in a stepwise fashion: a high-energy particle must pass near enough to the strand so that the ions and radicals produced along its trajectory are close enough to interact with the strand, and a radical in the vicinity of the DNA molecule actually interacts with a critical site on the molecule and induces a SSB. Over time, the SSB damage accumulates, and DNA molecules in a trapped population begin to suffer DSB. The authors seek to determine the fraction of trapped DNA that survives after an interval of time subject to a specified flux of ionizing radiation.

First, the effects of the accumulation of SSB will be examined. Later, in §6, the effects of single-track DSB will be included. In the model described by the authors, lengths are most conveniently measured in units of base pairs, each contributing a length b to a DNA strand.

3. SSB accumulation

The formation of a SSB on one strand of a DNA molecule creates a critical zone on the opposite strand that cannot sustain another SSB and, as a consequence, produce a DSB. As mentioned in the

previous section, this zone extends from $b=2.64$ to about $b=10$ base pairs up and down the opposite strand. In a DNA molecule L base pair units long, the probability that the first SSB in the second strand avoids the critical zone is $[L-(2b+1)]/L$. The probability that a second SSB produces no DSB is then $[L-2(2b+1)]/(L-1)$, and for the n th SSB, $[L-n(2b+1)]/(L-n+1)$. Therefore, the probability that all the SSB in the second strand avoid the critical regions is

$$\prod_{i=1}^n \frac{L-i(2b+1)}{L-i+1} \quad (1)$$

If we assume that, on average, each of the two strands suffers half the total number of SSB on the molecule, then with n SSB forming on one strand the total number of SSB on both is $\mathcal{N}=2n$. In the situations the authors will be considering n is small compared with L , and this product can be approximated by

$$\left[\frac{L-n(2b+1)}{L} \right]^n \quad (2)$$

Thus, the fraction $F(\mathcal{N})$ of DNA molecules which suffer \mathcal{N} SSB distributed equally among the two strands of the helix and possess no DSB is (cf. analysis in Freifelder and Trumbo 1969)

$$F(\mathcal{N}) = \left[\frac{L-n(2b+1)}{L} \right]^n = \left[\frac{L-\mathcal{N}/2(2b+1)}{L} \right]^{\mathcal{N}/2} \quad (3)$$

This transcendental equation needs to be solved for \mathcal{N} so that once a survival rate is specified the average number of SSB that a DNA molecule can sustain and not produce a DSB can be determined.

$$\begin{aligned} \ln F &= \frac{\mathcal{N}}{2} \ln \left[1 - \frac{\mathcal{N}}{2L}(2b+1) \right] \\ &\approx -\frac{\mathcal{N}}{2} \left(\frac{\mathcal{N}}{2L}(2b+1) \right) = -\frac{2b+1}{4L} \mathcal{N}^2 \end{aligned} \quad (4)$$

and thus

$$\mathcal{N} = \left[-\frac{4L \ln F}{2b+1} \right]^{1/2} \quad (5)$$

where the Taylor approximation that $\ln(1-x) \approx -x$ has been used. Table 1 lists the number of SSB that can be sustained for various size genomes and for varying values of the parameter b and have a fraction F of the population survive without a DSB. The sizes

Table 1. Average number of SSB that a genome of length L in base pairs can sustain so that a fraction F of a trapped population will survive without a lethal DSB. The number of base pairs b between two SSB that are needed to avoid a DSB varies with the ionic strength of the medium surrounding the DNA molecule.

b	$F=10^{-2}$			$F=10^{-4}$		
	L			L		
	10^6	5×10^6	10^7	10^6	5×10^6	10^7
2.64	1700	3800	5400	2400	5400	7700
5	1300	2900	4100	1800	4100	5800
10	940	2100	3000	1300	3000	4200
20	670	1500	2100	950	2100	3000

of DNA genomes for bacteria vary from about 760 000 base pairs in mycoplasma to about 5 million in *Escherichia coli*. The minimal size genome for cellular life is approximately 320 000 base pairs (Mushegian and Koonin 1996). For the case of DNA in media of high ionic strength, such as is the case for the halotolerant bacteria, several thousand SSB can form and still yield an appreciable survival rate.

4. Interaction probabilities

Although the linear energy transfer (LET) model is useful when considering the stopping power of various media, it proves to be inadequate when analyzing the interaction with specific structures the size of a DNA molecule. Ionizing radiation produces aggregates of ions along its path. These energy deposition events vary in frequency and size from spurs 4 nm in diameter, which account for approximately 95% of the events and about 80% of the deposited energy, to larger aggregates about 7 nm in diameter and accounting for 5% of the events and roughly 20% of the energy deposited. A spur forms from a loss of less than 100 eV; the larger aggregates form from depositions of approximately 500 eV or less (Ward 1988). Furthermore, these energy deposition structures are well separated by approximately 400 nm (Chatterjee and Magee 1985). Thus, in considering whether radiation can induce damage to a particular DNA molecule, the authors take up an analysis of the probability that the trajectory takes the particle close enough to the molecule *and* a spur or cluster forms near enough to interact.

In pure water, the ions created as part of a spur or cluster can diffuse many tens of nanometers away from the sites of their creation in a time equal to the half-life of the reactions involving the radicals (Turner 1995). In a typical fluid inclusion such as being considered, the solute ions present act as effective

scavengers. The half-life of such scavenger reactions is $\tau_{\text{scav}} = (\ln 2)/K(S)$, where $K(S)$ is the reaction rate for solute species S with the relevant radicals and ions. Typically, the rate constant is $10^9 \text{ M}^{-1} \text{ s}^{-1}$. In a time τ_{scav} , a radical will diffuse a distance λ given by $\lambda = (6\tau_{\text{scav}}D)^{1/2}$, where D is the diffusion constant for the particular species of radical. D varies from $2 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ for $\text{OH}\cdot$, one of the more reactive species, to $8 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ for H_3O^+ . A saturated NaCl brine is 6 M. In eubacteria that form spores, a high level of compensatory osmoregulatory material will be produced to offset the effects of the brine in which the spore is immersed. This material will generally bind water strongly to its structure. Examples of such regulatory materials are ectoine, hydroxyectoine or glycine-betaine (Galinski 1993, Del Moral *et al.* 1994). The spores also tend to dehydrate themselves by primarily removing the osmotically responsive water, leaving mostly the bound water. With these effects, the intraspore environment can be approximated as equivalent to a 6 M brine in terms of its ability to generate, diffuse and scavenge ions and radicals. With this assumption, $\tau_{\text{scav}} \approx 10^{-10} \text{ s}$. This value is consistent with studies of molecular yields of water radiolysis showing that high concentrations of scavengers reduce the availability of ions beyond the spur (Schwarz 1955). These data also imply that intraspur reactions among the radicals are heavily suppressed. A 0.3 M concentration of bromide ions yields half the hydrogen peroxide with this ion's presence. At this concentration the OH radicals possess a half-life of $2 \times 10^{-9} \text{ s}$, consistent with the τ_{scav} of about 10^{-10} s for a higher concentration of solute ions. It is also noted that these time scales arise in the context of *in vivo* studies of Chinese hamster cells. Reuvers *et al.* (1973) demonstrate the protective action of four different OH scavengers and conclude that the half-life of this radical is approximately 10^{-9} s . A value of $\tau_{\text{scav}} \approx 10^{-10} \text{ s}$ implies $\lambda \approx 2 \text{ nm}$. This figure illustrates that the radicals do not possess a large diffusive range in the context being considered. Thus, the trajectory of a high-energy particle needs to pass within a distance $\lambda \approx 2 \text{ nm}$ on either side of a DNA strand in order to interact with it.

First to be determined is the probability that the trajectory of a randomly emitted particle originating within the inclusion volume V_{inc} approaches close enough for radicals to diffuse over and induce a lesion. A DNA molecule constructed of L base pairs will have a length L (3.4 nm/10 base pairs) and a width of 2 nm. (Supercoiling is ignored, which will only serve to lessen the cross-section for interaction.) If such a naked strand is considered to be the target of the radiation as in figure 1, then the probability

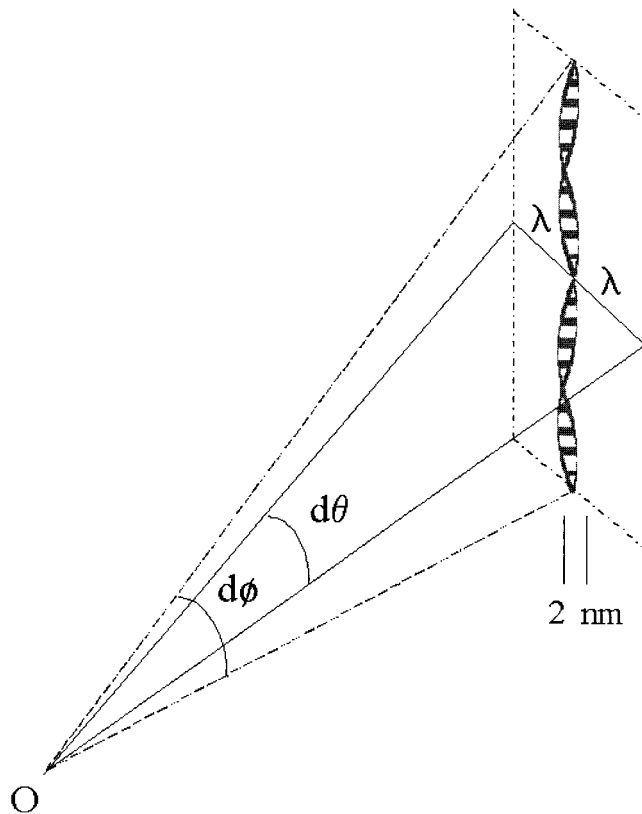


Figure 1. Geometrical configuration of a radionuclide at O which can emit a high-energy particle that might interact with a naked DNA strand. In order to interact with the strand, the particle must pass within a distance λ on either side of the strand.

that its path passes within a distance λ on either side of it is

$$P_{\text{traj}} = P_{\theta} P_{\phi} = \left(\frac{d\theta}{2\pi}\right) \left(\frac{d\phi}{2\pi}\right) = \left[\frac{1}{2\pi} \frac{2\lambda + 2 \text{ nm}}{x_{\text{avg}}}\right] \times \left[\frac{1}{2\pi} \frac{L(3.4 \text{ nm}/10 \text{ base pairs})}{x_{\text{avg}}}\right] = \frac{2\lambda + 2 \text{ nm}}{4\pi^2 x_{\text{avg}}^2} L \left(\frac{3.4 \text{ nm}}{10 \text{ base pairs}}\right) \quad (6)$$

where

$$\lambda = \left(\frac{6D \ln 2}{K(S)}\right)^{1/2} \quad (7)$$

and x_{avg} is the average separation between the DNA strand and all other points in the volume of the inclusion. That is, for a DNA molecule located at an arbitrary point (x_0, y_0, z_0) ,

$$x_{\text{avg}} = \frac{1}{V_{\text{inc}}} \iiint_{V_{\text{inc}}} [(x-x_0)^2 + (y-y_0)^2 + (z-z_0)^2]^{1/2} dx dy dz \quad (8)$$

For arbitrary values of (x_0, y_0, z_0) , this integral cannot be evaluated in closed form, but it can be approximated by

$$x_{\text{avg}} = \frac{3}{4} V_{\text{inc}}^{1/3} \quad (9)$$

The fact that the spurs and other aggregates of radicals and ions are separated by approximately 400 nm implies that the probability that one of these ion structures forms along the trajectory and is near enough to interact with the DNA molecule is

$$P_{\text{ion proximity}} = P_{\text{traj}} \left(\frac{2\lambda + 2 \text{ nm}}{400 \text{ nm}} \right) \quad (10)$$

where the fact is used that the spur or aggregate of ions can form either a distance λ in front of the 2 nm wide DNA molecule or a distance λ behind it. This expression can be refined if the energies of the particles involved are known. Specifically, the 400 nm separation between the energy deposition structures is simply a typical figure for high-energy electrons. It is assumed that the energy of a particle is lost quasi-continuously over its entire path in the form of spurs and aggregates. The weighted energy content per structure is $0.8(100 \text{ eV}) + 0.2(500 \text{ eV}) = 180 \text{ eV}$. Thus, if R_0 is the range of a particle of energy E , the average distance between energy deposition structures is

$$S = \frac{R_0}{E} \frac{180 \text{ eV}}{1 \text{ structure}} \quad (11)$$

For betas, the range in cm for a particle of energy E in MeV moving through a medium of density ρ in g/cm^3 is (Turner 1995)

$$R_0 = \frac{0.412}{\rho} E^{1.27 - 0.0954 \ln E} \quad (12)$$

Therefore, the average distance between structures becomes

$$S = \frac{0.412}{\rho} E^{0.27 - 0.0954 \ln E} \left(\frac{1.8 \times 10^{-4} \text{ MeV}}{1 \text{ structure}} \right) \quad (13)$$

where E is in MeV, ρ in g/cm^3 , and S in cm. Because the weighted energy content per structure is approximately 180 eV and approximately 31 eV are required to produce an ion pair (ICRU 31 1979), on average, energy deposition structures contain about five ion pairs. These five ions are contained somewhere in the nearly cylindrical volume of

$$V_{\text{ions}} = \pi(\lambda + 1 \text{ nm})^2 \lambda \quad (14)$$

Most of the volume contains scavengers and fluid; the DNA molecule itself occupies only a small fraction of the volume wherein the ions possibly have been created. The probability that the ions react with the

DNA is

$$P_{\text{DNA react}} = 5 \frac{\pi(1 \text{ nm})^2 \lambda}{\pi(\lambda + 1 \text{ nm})^2 \lambda} = 5 \frac{1 \text{ nm}^2}{(\lambda + 1 \text{ nm})^2} \quad (15)$$

Further, even if the radicals interact with the DNA molecule, the damage induced may not be at a critical site, i.e. a phosphodiester bond, and yield a SSB. The ratio of base damage to sugar damage in double-stranded DNA is about 2.7 (Ward 1985), accounting for both damage caused from diffusing OH radicals and direct ionization. This ratio, though, has been measured to be as high as 7 to 10 (Ward and Kuo 1978). Thus, even if a radical interacts with a portion of the DNA molecule, the probability that it produces an SSB rather than base damage is reduced by a factor of at least 2.7. Therefore, of the high-energy particles whose trajectories take them near enough, only the fraction

$$\begin{aligned} & \left(\frac{2\lambda + 2 \text{ nm}}{S} \right) \left(\frac{5 \text{ nm}^2}{(\lambda + 1 \text{ nm})^2} \right) \left(\frac{1}{2.7} \right) \\ &= \frac{2}{S} \left(\frac{1 \text{ nm}^2}{\lambda + 1 \text{ nm}} \right) \left(\frac{1}{2.7} \right) \end{aligned} \quad (16)$$

will induce a SSB, assuming that any damage to a phosphodiester moiety always results in a SSB. Finally, the probability that a randomly emitted particle will pass a DNA molecule and induce a SSB is

$$\begin{aligned} P_{\text{SSB}} &= \frac{2(\lambda + 1 \text{ nm})}{4\pi^2 \left(\frac{3}{4} V_{\text{inc}}^{1/3} \right)^2} L \left(\frac{3.4 \text{ nm}}{10 \text{ base pairs}} \right) \\ &\times \frac{2}{S} \left(\frac{5 \text{ nm}^2}{\lambda + 1 \text{ nm}} \right) \frac{1}{2.7} \end{aligned} \quad (17)$$

which, in a convenient set of units, reduces to

$$P_{\text{SSB}} = 1.12 \times 10^{-19} \text{ mm}^3 \frac{L}{S V_{\text{inc}}^{2/3}} \quad (18)$$

where L is measured in base pairs and the volume of the inclusion V_{inc} is measured in cubic millimeters. S , the average separation between energy deposition structures, is now in millimeters.

The striking feature of this result is that it is independent of the average diffusion distance of the radicals and ions. This feature of the result can be understood by noting that a large diffusion distance λ means that a spur or aggregate can form further away from the DNA strand and still interact with it. But, because the spur or aggregate contains a fixed number of ions, those ions are diluted over a larger volume. The two effects compensate, and the probability of interaction is independent of λ .

Note that if the size of the inclusion is large compared to the average range R of particles emitted

from radionuclides uniformly dispersed within the inclusion volume V_{inc} , P_{SSB} needs to be reduced by a form factor of the order of R^3/V_{inc} . This accounts for a reduction in particle flux due to attenuation produced by the fluid itself. The volume accessible to damage may be substantially smaller than V_{inc} . For example, $^{40}_{19}\text{K}$ betas have a maximum energy of 1.312 MeV and an average energy of 0.585 MeV. The ranges of such betas in a 1.2 g/cm^3 brine are 0.46 cm and 0.20 cm, respectively. Betas typically penetrate only to about one half of their range for energies up to about 1.25 MeV (Turner *et al.* 1988). Thus, on average, betas originating from nuclei more distant than 0.1 cm do not reach a DNA strand. Even though a bacterial spore may be physically confined to an inclusion of volume V_{inc} , effectively it is subject to genetic damage from the radiation originating within a volume $V_{\text{eff}} = f(4/3)\pi R^3$, where R is the average range of any relevant high-energy particle and f is a factor of order unity which accounts for the relative location of DNA within V_{inc} . For example, if a spore is located in one corner of a cubic inclusion, only one-eighth of the fluid volume produces particles that can damage the spore DNA.

5. Survival time limited by SSB accumulation

Let a survival time T_F be the average time interval after entrapment that a fraction F of a confined population of spores survives. The probability has been evaluated that the DNA of such spores suffer SSB due to the presence of ionizing radiation, and the average number of SSB that a DNA strand can suffer and not sustain a DSB has been determined. It remains to consider the rate at which ionizing events occur. As seen, this rate is intimately dependent on the nature and concentration of radioactive nuclei in the environment and the geometric configuration of the entrapment.

Consider a fluid inclusion containing a beta emitter, such as $^{40}_{19}\text{K}$, as a solute ion at a present concentration of $C\text{ g/mm}^3$. The total number of nuclei with gram-molecular-mass M is $N_n = CV_{\text{inc}}N_A/M$. These nuclei remain from an original number N_0 at entrapment, and $N_n = N_0 \exp\{-(\ln 2)t/t_{1/2}\}$. Thus, $N_0 = N_n \exp\{(\ln 2)t/t_{1/2}\}$. And the number of decays that have occurred over this time t is

$$\begin{aligned} N_0 - N_n &= N_0 \{1 - \exp[-(\ln 2)t/t_{1/2}]\} \\ &= N_n \exp[(\ln 2)t/t_{1/2}] \{1 - \exp[-(\ln 2)t/t_{1/2}]\} \\ &= N_n \{\exp[(\ln 2)t/t_{1/2}] - 1\} \\ &= \frac{CV_{\text{inc}}N_A}{M} \{\exp[(\ln 2)t/t_{1/2}] - 1\} \end{aligned} \quad (19)$$

The number of these decays that will produce SSB after a time T_F will be

$$\begin{aligned} N_{\text{SSB}} &= \frac{CV_{\text{inc}}N_A}{M} \{\exp[(\ln 2)T_F/t_{1/2}] - 1\} \\ &\quad \times (1.12 \times 10^{-19}\text{ mm}^3) \frac{L}{SV_{\text{inc}}^{2/3}} \\ &= (1.12 \times 10^{-19}\text{ mm}^3) \frac{CV_{\text{inc}}^{1/3} L N_A}{S M} \\ &\quad \times \{\exp[(\ln 2)T_F/t_{1/2}] - 1\} \end{aligned} \quad (20)$$

Solving this expression for T_F yields

$$\begin{aligned} T_F &= \frac{t_{1/2}}{\ln 2} \left[\frac{SN_{\text{SSB}}M}{(1.12 \times 10^{-19}\text{ mm}^3)CV_{\text{inc}}^{1/3} N_A L} \right] \\ &= \frac{t_{1/2}}{\ln 2} \left[-\frac{4 \ln F}{2b+1} \right]^{1/2} \\ &\quad \times \left[\frac{SM}{(1.12 \times 10^{-19}\text{ mm}^3)CV_{\text{inc}}^{1/3} N_A L^{1/2}} \right] \end{aligned} \quad (21)$$

where used again is the Taylor series expansion $\ln(1+x) \approx x$, for x small compared to 1.

6. Effect of single-track DSB and total surviving fraction

Not considered so far is the effect of single-track DSB. Such a DSB will arise if the particle track lies roughly along a line formed by a base pair or the interstitial regions between successive base pairs; other trajectories will result in a SSB.

The relative probability that a single track results in a DSB rather than a SSB can be estimated. One helical segment of a DNA strand is 3.4 nm in length and 2 nm in width, representing a total target area of 7 nm^2 . The diameter of a base pair region is approximately 0.6 nm, presenting a cross-sectional target area of 0.3 nm^2 . Thus, $0.3/7 = 0.04$ of the particle interactions ought to yield a DSB rather than a SSB. In fact, this heuristic result is confirmed experimentally. For the human genome, the rate of formation of DNA SSB is 1000 per Gy and 40 single-track DSB per Gy (Ward 1988), or equivalently, a DSB rate of $10^{-2}\text{ DSB Gy}^{-1}\text{ Mbp}^{-1}$. Thus, to calculate the number of DSB produced in a population of N_{pop} individuals, the dose to which the population has been subjected needs to be determined.

This dose is dependent upon the properties of the relevant radionuclide, and for spores trapped in halite inclusions $^{40}_{19}\text{K}$ is the most likely source of high-energy particles. This radionuclide produces

betas of average energy 0.585 MeV/decay or 9.37×10^{-14} J/decay. (It also emits gammas, a circumstance that will be addressed in §7.) The number of decays after a time t is given by equation 19, where the concentration of radionuclides is C (in g/mm^3) and V_{inc} is the volume of the inclusion (in mm^3). M is the gram-molecular-mass of the radionuclide, 40 in the present case, and N_A is Avagadro's number. Thus, for a fluid of density ρ (in g/mm^3) within the inclusion, the dose will be

$$D = \frac{CV_{\text{inc}}N_A}{M} \{ \exp [(\ln 2)t/t_{1/2}] - 1 \} (9.37 \times 10^{-14}) \text{J}$$

$$= \frac{CN_A}{M\rho} \{ \exp [(\ln 2)t/t_{1/2}] - 1 \} (9.37 \times 10^{-8}) \text{Gy}$$
(22)

where now ρ is in g/cm^3 . For a DSB rate of 10^{-2} DSB $\text{Gy}^{-1} \text{Mbp}^{-1} = (10^{-8} \text{ DSB Gy}^{-1} \text{bp}^{-1})L$, the number of single-track DSB is given by

$$N_{\text{DSB, single-track}}^{K-40} = \frac{CN_A L}{M\rho} (9.37 \times 10^{-16})$$

$$\times \{ \exp [(\ln 2)t/t_{1/2}] - 1 \}$$
(23)

For a trapped population to yield individuals that suffer no single-track DSB, the number in the population needs to exceed the number of single-track DSB induced. Simply being roughly equal or marginally larger will not suffice, for additional individuals will accumulate enough SSB to form fatal DSB.

The number of single-track DSB, of course, will not be distributed uniformly among the individuals, but instead will follow a Poisson distribution. For an average occurrence of

$$\mu = N_{\text{DSB, single-track}}^{K-40} / N_{\text{pop}}$$
(24)

the probability that an individual in the population will sustain k single-track DSB is

$$P(k) = \frac{\mu^k}{k!} e^{-\mu}$$
(25)

$k = 0, 1, 2, \dots$ and the probability that an individual suffers no single-track DSB is $P(0) = e^{-\mu}$.

Spores will be killed by two mechanisms acting simultaneously: single-track DSB and the accumulation of enough SSB to form a DSB. A segment of the trapped population will suffer both types of DSB. Thus, when the number of SSB as a function of time is given by equation 20 and the corresponding surviving fraction is given by equation 5, the fraction of surviving spores that have not suffered a DSB of any type is

$$F_{\text{Total}} = F e^{-\mu}$$
(26)

Note that both F and μ are functions of time. Figure 2 illustrates this time-dependence. Note that for very early times ($t < 0.4$ Ga) single-track DSB is the dominant killing mechanism, but eventually the accumulation of SSB produces more DSB than those which arise from single tracks. For increasingly smaller inclusions trapping progressively fewer spores, single-track DSB dominate as the principal source of genetic damage for increasingly larger fractions of the survival time. Note, also, that the time-dependence of the surviving fraction is basically that of the accumulation of SSB, but diminished to a small but significant extent by the effects of single-track DSB. The concentration of the radionuclide is by far the most influential factor limiting survival time. Figure 3 shows the total fraction of surviving spores F_{total} as a function of time for four concentrations of $^{40}_{19}\text{K}$. Although less dramatic, the dependence on b nonetheless is significant (b is the minimum number of base pairs that can retain the integrity of the two strands of DNA without a DSB forming.) Figure 4 illustrates the dependence of the survival rate on b . Finally, the surviving fraction depends also on the number of trapped individuals, and figure 5 illustrates that relationship. For populations in excess of about 50 000 individuals, the surviving fraction becomes nearly independent of the size of the trapped population.

Determining the number of viable spores in a sample extracted from an inclusion would be difficult to ascertain experimentally. More to the point, once an inclusion has been drained and any trapped spores extracted, only a few viable survivors are needed to

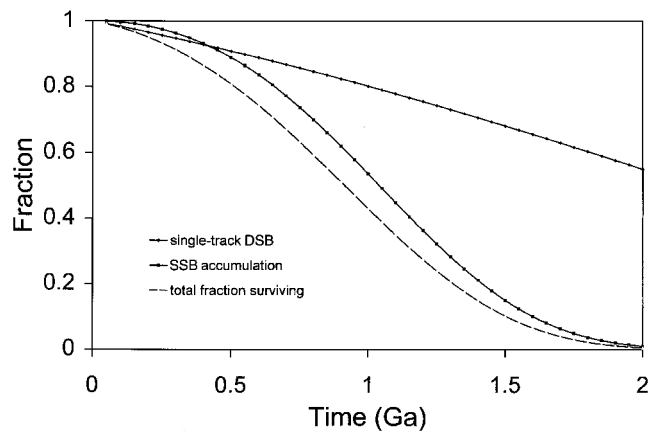


Figure 2. Relative effects of single-track DSB and DSB produced by the accumulation of SSB for an entrapped population of 10 000 individuals whose genome has a length of 4 Mbp and is subject to damage by $^{40}_{19}\text{K}$ betas when this radionuclide is present at a concentration of $6.5 \times 10^{-11} \text{g}/\text{mm}^3$. For these calculations, we have chosen $b = 2.64$, the smallest experimentally determined value (Freifelder and Trumbo 1969). 1 Ga = 10^9 years.

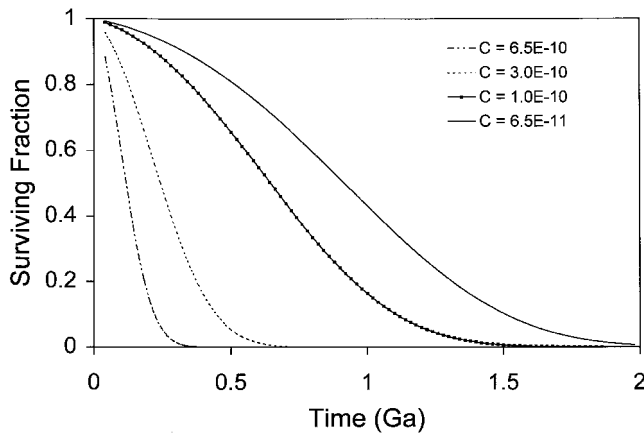


Figure 3. The dependence of survival time on the concentration (in g/mm^3) of the radionuclide ^{40}K trapped with the spores. For survival times for small fractions of surviving spores, see table 2. These calculations assume a trapped population of 10 000 and a genome length of 4 Mbp. b was chosen to be 2.64 again.

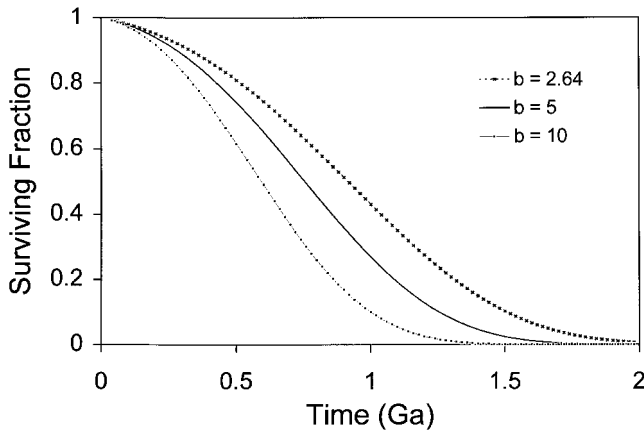


Figure 4. The effect of b on survival time. b is the number of base pairs that are needed between two SSB on opposite strands so that a DSB does not form. The size of the trapped population is 10 000, and the size of the genome is 4 Mbp. The concentration of ^{40}K is $6.5 \times 10^{-11} \text{ g}/\text{mm}^3$.

successfully culture the sample. In table 2, the time intervals T_F for three fractional survival rates are quoted: $F = 10^{-2}$, 10^{-4} and 10^{-6} . Values of $T_{10^{-6}}$ are given to better facilitate comparisons with previously published models and data, even though trapping spore populations in the millions is unlikely.

7. Discussion

We have considered limitations to the survival times of a trapped population of spores subject to the action of beta radiation originating within the inclusion trapping the spores. Such a circumstance subjects the spores to a low physical dose rate seldom investigated. In fact, the physical dose rate to which

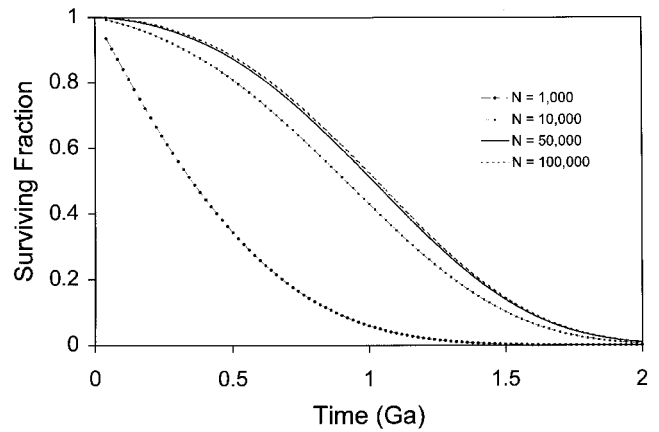


Figure 5. The dependence of the surviving fraction of spores on the number of trapped individuals. For populations exceeding about 50 000 individuals, the surviving fraction is nearly independent of the number of spores initially trapped within the inclusion. For these calculations, $b = 2.64$ and the concentration of ^{40}K is $6.5 \times 10^{-11} \text{ g}/\text{mm}^3$.

Table 2. Survival times for three fractions of a population of trapped spores and their dependence on the concentration of ^{40}K and the number of base pairs needed to prevent DNA strands from denaturing.

C (g/mm^3)	b (base pairs)	Survival times		
		$T_{10^{-2}}$ (Ga)	$T_{10^{-4}}$ (Ga)	$T_{10^{-6}}$ (Ga)
6.5×10^{-9}	2.64	0.31	0.43	0.53
	5	0.24	0.34	0.41
	10	0.18	0.25	0.30
3.0×10^{-10}	2.64	0.61	0.83	0.98
	5	0.49	0.67	0.79
	10	0.37	0.51	0.61
1.0×10^{-10}	2.64	1.4	1.8	2.1
	5	1.2	1.5	1.8
	10	0.94	1.1	1.4
6.5×10^{-11}	2.64	1.9	2.4	2.7
	5	1.6	2.0	2.3
	10	1.3	1.7	1.9

The data are for an initial population of 10 000 individuals whose genome length is 4.0 Mbp trapped in an inclusion whose volume is 9 mm^3 and with a brine of density $1.2 \text{ g}/\text{cm}^3$.

the trapped spores are exposed is somewhat less than the background dose rate encountered at the earth's surface. As shall shortly be argued, at the depth in which a typical halite inclusion is located, the cosmic ray flux is greatly attenuated, and alpha sources are effectively excluded from halite crystals and pose an inconsequential threat for genetic damage.

As part of a comprehensive study examining the natural transfer of viable microbes in space, Mileikowsky *et al.* (2000) used a track structure model (based on Katz *et al.* (1971) and Cucinotta *et al.*

(1995)) to calculate survival times until $N/N_0 = 10^{-6}$ for *B. subtilis* (wild-type and pol^-) and *Deinococcus radiodurans* shielded against the galactic cosmic ray flux by modeled Martian regolith. The smallest physical dose rate presented is 0.06 cGy/a. We seek to compare their model with the one presented here.

As seen, the dose rate depends primarily on the concentration of $^{40}_{19}\text{K}$. The concentration of potassium, and consequently, this radionuclide, varies throughout the strata associated with the Permian Salado Formation from where strain 2-9-3 was extracted. For concentrations of $^{40}_{19}\text{K}$ of $6.5 \times 10^{-10} \text{ g/mm}^3$ down to $6.5 \times 10^{-11} \text{ g/mm}^3$, the corresponding physical dose rates are $7.9 \times 10^{-3} \text{ cGy/a}$ and $7.9 \times 10^{-4} \text{ cGy/a}$, respectively. Extrapolating the results of Mileikowsky *et al.* (2000) to an even lower dose rate than 0.06 cGy/a yields survival times until $N/N_0 = 10^{-6}$ of 180 Ma for a dose rate of $7.9 \times 10^{-3} \text{ cGy/a}$ and 1.7 Ga for $7.9 \times 10^{-4} \text{ cGy/a}$ for *B. subtilis*. The corresponding times for *D. radiodurans* are approximately a factor of 3 longer. A comparison of these extrapolations with results in table 2 makes it apparent that they agree to about a factor of 2. Typically, this level of indeterminacy is characteristic of track structure calculations. The extrapolation of the model in Mileikowsky *et al.* (2000) to such a low dose rate is dangerous, but at least it shows that their model is consistent with the direct representation presented here. Both approaches indicate that for the low dose rates to which trapped spores such as strain 2-9-3 have been exposed, survival times are measured in hundreds of millions of years.

The efficacy of the model may also be compared with measured rates of induced damage to DNA. Newman *et al.* (1997) irradiated Chinese hamster V79 cells with alphas from ^{238}Pu which possessed an incident energy at the cells of 3.5 MeV and corresponded to an LET of 110 keV/ μm . The range of these alphas within the cell is 31.8 μm , and using equation 11 the average distance S between energy deposition structures is $1.6 \times 10^{-6} \text{ mm}$. From the authors' model, the number of SSB induced per Gy per bp is given by equation 20 divided by equation 22 and divided by L , i.e.

$$(1.12 \times 10^{-19} \text{ mm}^3) V_{\text{inc}}^{1/3} \rho / S,$$

which for this case is $5.7 \times 10^{-6} \text{ SSB Gy}^{-1} \text{ bp}^{-1}$. Table 1 indicates that, depending on the size of the genome, b , and the surviving fraction of spores, roughly 2000 SSB need to accumulate so as to form a DSB. The 4% contribution of single-track DSB is not significant at this level of approximation. Thus, we expect a DSB formation rate of approximately $3 \times 10^{-9} \text{ DSB Gy}^{-1} \text{ bp}^{-1}$. This rate agrees with

the value of $3.15 \pm 0.29 \times 10^{-9} \text{ DSB Gy}^{-1} \text{ bp}^{-1}$ for alphas measured by Newman *et al.* (1997) using the distribution size method (Lehmann and Ormerod 1970). This agreement is, to some extent, fortuitous and coincidental, for the present model would have predicted values a factor of 2 higher and lower than this depending on the values of b , L , and F . Using the model of Cook and Mortimer (1991), Newman *et al.* (1997) find for alphas that the breakage frequencies ranged from $4.5 \pm 0.8 \times 10^{-9} \text{ DSB Gy}^{-1} \text{ bp}^{-1}$ for fragments with a mean weight of 5 Mbp to $633 \pm 140 \times 10^{-9} \text{ DSB Gy}^{-1} \text{ bp}^{-1}$ for fragments of 14 kbp mean weight. The agreement with the present authors' model is still satisfying.

The results of the present model can be checked for consistency with the observed survival of bacterial spores trapped for 250 Ma. In Vreeland *et al.*, the spores were extracted from an inclusion $3 \times 3 \times 1 \text{ mm}$. The threat to the genome in this case arises from the $^{40}_{19}\text{K}$ in the brine; potassium would largely be excluded from the solid crystal. The concentration of potassium in the brine is approximately 6.2 g/l = $6.2 \times 10^{-6} \text{ g/mm}^3$. This figure represents a concentration of potassium averaged over all the strata comprising the Permian Salado Formation. The $^{40}_{19}\text{K}$ abundance is 0.0117% of the natural potassium abundance. Further, only 89.3% of the decays of this radionuclide produce betas ($E_{\text{max}} = 1.312 \text{ MeV}$; $E_{\text{avg}} = 0.585 \text{ MeV}$); the remainder decay by electron capture accompanied by the emission of a 1.461 MeV gamma. The concentration of beta emitters is therefore $6.5 \times 10^{-10} \text{ g/mm}^3$. With an average beta energy of 0.585 MeV, the average separation S of the energy deposition structures is $5.2 \times 10^{-4} \text{ mm}$ (520 nm). From equation 20 the survival time for $F = 1\%$, $b = 2.64$ base pairs, and $L = 4 \times 10^6$ base pairs is $T_{1\%} = 3.1 \times 10^8 \text{ a}$, which is consistent with the observed survival of *Bacillus* strain 2-9-3 over 250 Ma.

Although a genome length of $L = 4 \times 10^6$ base pairs was used for strain 2-9-3, the actual value has not been determined. The actual length, though, is likely to be somewhat smaller than the value used, which is equivalent to the size for *E. coli*. A smaller value for L would produce a larger value for T_F .

As can be seen from equation 20, T_F is a weak function of F (increasing as $(\ln F)^{1/2}$) and also of L (decreasing as $L^{1/2}$). The most important factor to the survival of bacterial DNA, however, is the concentration of the radionuclides. The value of C at the location of the sample used by Vreeland *et al.* is likely one full order of magnitude smaller than what was used above. With a concentration of emitters of $6.5 \times 10^{-11} \text{ g/mm}^3$, $T_{1\%} = 1.9 \times 10^9 \text{ a}$ and $T_{0.01\%} = 2.4 \times 10^9 \text{ a}$ under optimum conditions where only an average of 2.64 base pairs are needed to retain DNA

strand integrity. These times fall to 1.3×10^9 a and 1.7×10^9 a when 10 base pairs are needed. A hypothetical population of bacteria with the minimal genome size would have a survival time in excess of the age of the universe under these same conditions.

Alpha particles do not pose a threat for genetic damage to spores trapped in halite inclusions. High-Z elements likely to be alpha emitters are effectively excluded from the crystal structure of halite. Even for inclusions near rocks bearing uranium series minerals, the range for alphas with energies of approximately 4 MeV is less than 1 mm, and so penetrate only an inconsequential distance into halite strata.

The contribution to genetic damage made by high-energy photons has not explicitly been considered. Gammas with energies between 100 keV and 10 MeV attenuate primarily by Compton scattering in materials near unit density. The relevant attenuation and energy absorption coefficients are so low that simply on this basis alone we can discount any effects produced by gammas originating within V_{inc} . Moreover, the flux is also negligible. Any gammas originating in the surrounding solids attenuate exponentially with a characteristic length scale of $1/\mu$. In a medium of unit density, $\mu \approx 0.03 \text{ cm}^{-1}$. The circumstance that would lead to significant genetic damage from outside the inclusion would be to have a substantial source located within a few multiples of $1/\mu$. Naturally occurring potassium cannot provide such a source.

Lastly, whether cosmic rays significantly limit the lifetimes of trapped spores is considered. Cosmic rays, high-energy particles (mostly protons), are an omnipresent source of ionizing radiation. This source represents about 10% of the average annual effective dose to individuals in the USA from various radiation sources. The flux of cosmic rays at the earth's surface, including particles created in collisions of primary cosmic rays with atoms in the atmosphere, is $2 \times 10^{-6} \text{ W/m}^2$. The flux of primary cosmic rays with energies above E GeV in the range 10^{-10} – 10^6 GeV is approximately equal to $1.7 E^{-1.6}$ particles $\text{cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$. For $E > 10^6$ GeV, the exponent increases from 1.6 to 2.2 so that, for example, particles with energies $E > 10^7$ GeV strike an area of 1 m^2 about once per year, a completely negligible flux. And particles with lower energy simply cannot penetrate with any significant flux to the deep layers where spores are likely to be buried. For particles with energies of 10^6 GeV or less, the typical penetration distance of protons in crustal material is less than 1 m. Cosmic rays prove to be an important source of ionizing radiation when considering entrapments within meteorites (Mileikowsky *et al.* 2000), but in relevant cases of

spores trapped in fluid inclusions within deeply buried crystals, cosmic rays are negligible sources of genetic damage and do not severely limit survival times.

Acknowledgements

AJN thanks K. G. Inman for his valuable and penetrating perspectives and suggestions. RHV and WDR gratefully acknowledge the support for this work, which was partially funded by Grants EAR9714203 and EAR0085371 from the National Science Foundation's Life in Extreme Environments Program.

References

- BARENSEN, G. W., 1990, Mechanisms of cell reproductive death and shapes of radiation dose–survival curves of mammalian cells. *International Journal of Radiation Biology*, **57**, 885–896.
- CHATERJEE, A. and MAGEE, J. L., 1985, Theoretical investigation of the production of strand breaks in DNA by water radicals, *Radiation Protection Dosimetry*, **13**, 137–140.
- CAMERINI-OTERO, R. D. and HSIEH, P., 1995, Homologous recombination proteins in prokaryotes and eukaryotes. *Annual Review of Genetics*, **29**, 509–552.
- COOK, V. E. and MORTIMER, R. K., 1991, A quantitative model of DNA fragments generated by ionizing radiation, and possible experimental applications. *Radiation Research*, **125**, 102–106.
- CUCINOTTA, F. A., WILSON, J. W., KATZ, R., ATWELL, W., BADHUSER, G. D. and SHAVERS, M. R., 1995, Track structure and radiation transport model for space radiobiology studies. *Advances in Space Research*, **18**, 183–194.
- DEL MORAL, A., SEVERIN, J., RAMOS-CORMENZONA, A., TRUPER, H. G. and GALINSKI, E. A., 1994, Compatible solutes in new moderately halophilic isolates. *FEMS Microbiology Letters*, **122**, 165–172.
- EISEN, J. A. and HANAWAIT, P. C., 1999, A phylogenomic study of DNA repair genes, proteins, and processes. *Mutation Research*, **435**, 171–213.
- FRANKENBERG, D., FRANKENBERG-SCHWAGER, M., BLÖCHER, D. and HARBICH, R., 1981, Evidence for DNA double strand breaks as the critical lesions in yeast cells irradiated with sparsely or densely ionizing radiation under oxic and anoxic conditions. *Radiation Research*, **88**, 524–532.
- FRANKENBERG-SCHWAGER, M. and FRANKENBERG, D., 1990, DNA double-strand breaks: their repair and relationship to cell killing in yeast. *International Journal of Radiation Biology*, **58**, 569–575.
- FREIFELDER, D., 1965, Mechanism of inactivation of coliphage T7 by x rays. *Proceedings of the National Academy of Sciences of the United States of America*, **54**, 128–134.
- FREIFELDER, D. and TRUMBO, B., 1969, Matching of single-strand breaks to form double-strand breaks in DNA. *Biopolymers*, **7**, 681–693.
- FRIEBERG, E. C., WALKEN, G. C. and SIEDE, W., 1995, *DNA Repair and Mutagenesis* (Washington, D.C.: ASM Press).
- GALINSKI, E. A., 1993, Compatible solutes of halophilic eubacteria: molecular principles, water-solute interaction, stress protection. *Experientia*, **49**, 487–496.
- GLASUNOV, A. V., GLASER, V. M. and KAPULTSEVICH, Y. G.,

- 1989, Two pathways of DNA double-strand break repair in G1 cells of *Saccharomyces cerevisiae*. *Yeast*, **35**, 131–139.
- INTERNATIONAL COMMISSION ON RADIATION UNITS AND MEASUREMENTS, 1979, Average energy required to produce an ion pair, ICRU Report 31 (Washington, D.C.: International Commission on Radiation Units and Measurements).
- IVANOV, E. L., SUGAWARA, N., FISHMAN-LOBELL, J. and HABER, J. E., 1996, Genetic requirements for the single-strand annealing pathway of double-strand break repair in *Saccharomyces cerevisiae*. *Genetics*, **142**, 693–704.
- JACKSON, J. D., 1975, *Classical Electrodynamics*, 2nd edition (New York: John Wiley and Sons).
- KATZ, R. B., ACKERSON, B., HOMAYOONFAR, M. and SCHARMA, S. C., 1971, Inactivation of cells by heavy ion bombardment. *Radiation Research*, **47**, 402–425.
- LEHMANN, A. R. and ORMEROD, M. G., 1970, Double-strand breaks in DNA of a mammalian cell after x-irradiation. *Biochimica et Biophysica Acta*, **217**, 268–277.
- MILEIKOWSKY, C., CUCINOTTA, F. A., WILSON, J. W., GLADMAN, B., HORNECK, G., LINDEGREN, L., MELOSH, J., RICKMAN, H., VALTONEN, M. and ZHENG, J. Q., 2000, Natural transfer of viable microbes in space. *Icarus*, **145**, 391–427.
- MINGOT, F., JORCANO, J. L. and DAVILA, C. A., 1978, Local perturbation of the double helical DNA structure near single breaks. *Biochimica et Biophysica Acta*, **520**, 1–10.
- MUSHEGIAN, A. R. and KOONIN, E. V., 1996, A minimal gene set for cellular life derived by comparison of complete bacterial genomes. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 10 268–10 273.
- NEWMAN, H. C., PRISE, K. M., FOLKARD, M. and MICAHIEL, B. D., 1997, DNA double-strand break distributions in X-ray and α -particle irradiated V79 cells: evidence for non-random breakage. *International Journal of Radiation Biology*, **71**, 347–363.
- OLIVE, P. L., 1998, The role of DNA single- and double-strand breaks in cell killing by ionizing radiation. *Radiation Research*, **150** (Suppl.), S42–S51.
- PARKES, R. J., 2000, A case of bacterial immortality? *Nature*, **407**, 844.
- REUVERS, A. P., GREENSTOCK, C. L., BORSA, J. and CHAPMAN, J. D., 1973, Studies on the mechanism of chemical radioprotection by dimethyl sulphoxide. *International Journal of Radiation Biology*, **24**, 533.
- SACHS, R. K., HAHNFELD, P. and BRENNER, D. J., 1997, The link between low-LET dose–response relations and the underlying kinetics of damage production/repair/misrepair. *International Journal of Radiation Biology*, **72**, 351–374.
- SCHWARZ, H. A., 1955, The effects of solutes on the molecular yields in the radiolysis of aqueous solutions. *Journal of the American Chemical Society*, **77**, 4960–4964.
- SHINAGAWA, H., 1996, SOS response as an adaptive response to DNA damage in prokaryotes. *Experientia*, **77**, 221–235.
- TURNER, J. E., 1995, *Atoms, Radiation, and Radiation Protection*, 2nd edition (New York: John Wiley and Sons).
- TURNER, J. E., HAMM, R. N., SOULEYRETTE, M. L., MARTZ, D. E., RHEA, T. A. and SCHMIDT, D. W., 1988, Calculations for β dosimetry using Monte Carlo code (OREC) for electron transport in water. *Health Physics*, **55**, 741–750.
- VREELAND, R. H., ROSENZWEIG, W. D. and POWERS, D. W., 2000, Isolation of a 250 million-year-old halotolerant bacterium from a primary salt crystal. *Nature*, **407**, 897–900.
- WARD, J. F., 1985, Biochemistry of DNA lesions. *Radiation Research*, **104**, S103–S111.
- WARD, J. F., 1988, DNA damage produced by ionizing radiation in mammalian cells: identities, mechanisms of formation and reparability. *Progress in Nucleic Acid Research and Molecular Biology*, **35**, 95–125.
- WARD, J. F., 1990, The yield of DNA double-strand breaks produced intracellularly by ionizing radiation: a review. *International Journal of Radiation Biology*, **57**, 1141–1150.
- WARD, J. F. and KUO, I., 1978, Radiation damage to DNA in aqueous solution: a comparison of the response of the single stranded form with that of the double stranded form. *Radiation Research*, **75**, 278–285.

Copyright of International Journal of Radiation Biology is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.