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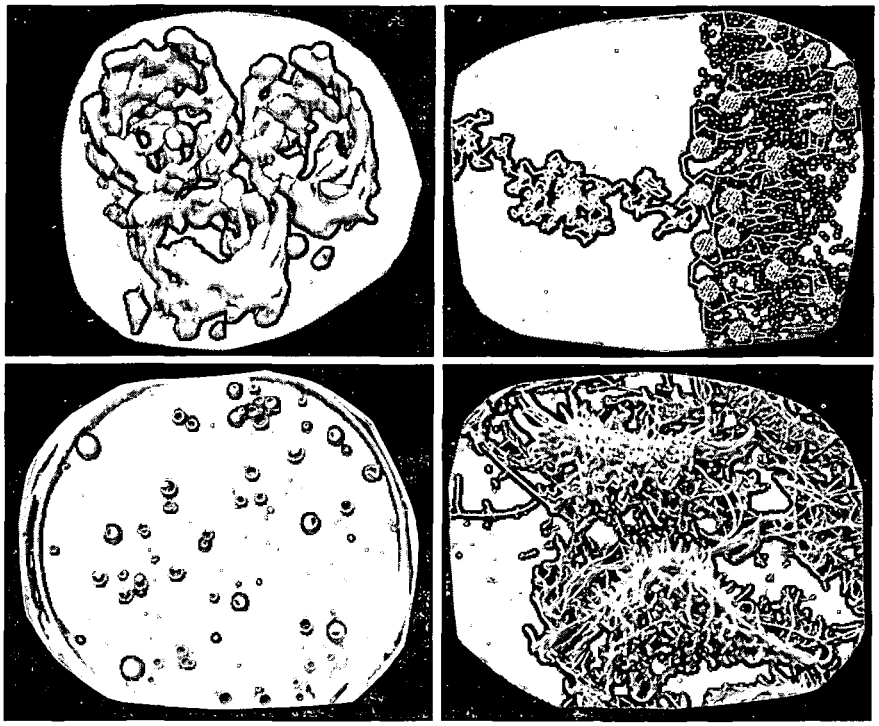

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Biochemical Mechanisms and Clusters of Damage for High-LET Radiation

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BIOCHEMICAL MECHANISMS AND CLUSTERS OF DAMAGE FOR HIGH-LET RADIATION

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ABSTRACT

Using mechanisms of indirect and direct radiation, a generalized theory has been developed to account for strand break yields by high-LET particles. The major assumptions of this theory are: (1) damage at deoxyribose sites results primarily in strand break formation and (2) damage to bases leads to a variety of base alterations. Results of the present theory compare well with cellular data without enzymatic repair. As an extension of this theory, we show that damage clusters are formed near each double strand break for high-LET radiation only. For 10 MeV/n (LET = 450 keV/μm) neon ions, the results show that on average there are ~3 additional breaks and ~3 damaged bases formed near each double strand break. For 100 MeV/n helium ions (LET = 3 keV/μm), less than 1% of the strand breaks have additional damage within 10 base pairs.

INTRODUCTION

Understanding the biochemical mechanisms associated with the production of DNA strand breaks by ionizing radiation is a subject of great interest. Considerable experimental effort by many investigators has been expended in such studies /1-4/, and, indeed, most of our present knowledge is due to the efforts of experimental radiation biochemists. For a better quantitative understanding these efforts need to be complemented by theoretical modeling in order to correlate energy loss events with the corresponding biochemical changes and eventually with the yields of strand breaks.

Most theoretical models of strand breaks tend to ignore the biochemical pathways. For example, Chariton and Humm /5/ have described a method for calculating DNA strand breaks induced by the decay of incorporated ^{125}I, and Goodhead and Nikjoo /6/ have analysed energy deposition of ultrasoft X-rays in target volumes with dimensions comparable to short sections of DNA. These calculations consider in some detail the track structure of low energy electrons but ignore the geometrical structure of DNA and many aspects of the biochemical stage.

Based on experimental data, the most important biochemical mechanisms associated with the production of strand breaks are: (1) water radical abstraction of H from the sugar rings (indirect mechanism) and (2) ionization of the sugar-phosphate backbone followed by deprotonation (direct mechanism). It has been reported in the literature /4/ that the average ·OH migration distance in a cellular environment is about 3.5 nanometers.

The theoretical model discussed here has the following features: (a) representation of a linear DNA molecule (in the B form) in a three-dimensional configuration based on X-ray diffraction data /7/, (b) simulation of the diffusive motion of water radicals in three dimensions, (c) reactions between sibling water radicals, (d) reactions of water radicals with scavengers, (e) reactions of water radicals with sugars and bases resulting in strand breaks (at sites of sugar damage) and base damage, respectively, and (f) ionization events on the DNA backbone leading to strand break formation. The details of these features have already been published /8-10/ and, hence, in this report we have only provided a brief review of each of them.

Consideration of track structure has played a significant role in the modeling effort, and has enabled us to calculate the yields of strand breaks for different qualities of radiation: photons, electrons, and heavy charged particles. High-LET radiation provides a useful probe for studying the different biochemical mechanisms in a systematic manner using selected track-segments.

Important features of the theoretical calculations are the variations of the yields of single and double strand breaks as a function of LET. In general, in our calculations we have defined double strand breaks as the occurrence of breaks on opposite strands with a maximum separation of 10 base pairs. We have compared the normalized yields of strand breaks (per unit Gray per unit dalton) as a function of linear energy transfer (LET) based on the theory with the corresponding experimental data measured in a cellular environment but with the inhibition of enzymatic repair. This comparison provides us with information on the validity of the biochemical mechanisms we have considered.

Our calculations have emphasized formation of DNA strand breaks since it appears likely that these lesions (in particular, double strand breaks) are responsible for most of the significant cellular effects (death, mutation, and malignant transformation) associated with ionizing radiation. It has been suggested that a certain class of double strand breaks is associated with a high concentration of damage over a relatively small volume. These clusters of damage may produce the most significant biological effects and be the most difficult to repair. High-LET radiation is likely to be much more effective in producing localized clusters of damage, and it is of great interest to have a quantitative estimate of the extent of this type of damage. In view of these considerations we have extended our theoretical model to incorporate the calculation of distributions of damaged sites in the vicinity of single and double strand breaks.

METHOD OF CALCULATION

The overall theoretical scheme has been developed in various stages which we now briefly describe.

Track Structure

Consideration of the track structure of energetic electrons is unavoidable under any irradiation condition with ionizing radiation. They are involved either as primary or secondary charged particles. Very low energy (~ 100 eV) as well as very high energy electrons (~ few MeV) should be included in any theoretical computation. In the present work, the concepts of track entities such as spurs, blobs, short tracks, and branch tracks have been used since there is a long history of their usefulness in radiation chemistry. These concepts and their applications in the Fricke dosimeter system to evaluate the ferric yields have been reported elsewhere /11/. Since the chemical mechanisms for DNA damage by indirect effects are qualitatively similar (·OH reactions with sugar sites), no change in the method of calculation was deemed necessary as far as track structure is concerned.

For heavy charged particles, the microscopic pattern of energy distribution is taken into account through the concept of "core" and "penumbra". Again, these concepts have previously been applied to the Fricke dosimeter system, and, for consistency, the procedure has remained the same for the DNA system. The radius, r_c, of a core is defined as the radius within which a major fraction of the glancing collisional losses are contained. In addition, the core contains the energy deposited by those low energy knock-on electrons which are stopped completely in this region. The radius of the core is given by $r_c = \hbar v/2\varepsilon_1$, where v is the velocity of the charged particle, ε_1 is the lowest electronic transition energy and $\hbar = h/2\pi$ is the modified Planck's constant. This radius is independent of the charge on the projectile but, of course, the magnitude of the energy density varies as the square of the charge.

For a 600 MeV/n particle, r_c = 82 Å, and when the energy decreases to 10 MeV/n this radius is 15 Å. The variation of r_c as a function of energy has been published elsewhere /12/. The region of energy deposition outside the core is called the penumbra. The average penumbra radius (in angstroms) is given by the empirical relation, $r_p = 396\ v^{2.7}$, where the charged particle velocity, v, is in units of 10^9 $cm.s^{-1}$. In the present work, each electron track in the penumbra has been considered independently.

Structure of DNA

In order to do the proper bookkeeping as to whether a given water radical has reacted with a base or a sugar, consideration of the DNA structure in three dimensions is essential. Only then can the accessibility of a given site (sugar or base) by a water radical be properly taken into account. It is known that the rate constants for ·OH reactions with isolated sugar or base molecules are about ten times greater than the corresponding average rate constants per sugar or per base in a structured DNA molecule. Hence, in the present calculations, it is not satisfactory to represent DNA by uniformly

distributed (or even randomly distributed) isolated sugar and base molecules at their average concentrations. Instead, the constituent molecules of the DNA polymer are positioned in Cartesian coordinates based on X-ray diffraction data /7/ and in this manner the locations of the sugar or base molecules which have undergone reactions can be accurately stored in the computer memory. From this information, one can easily determine whether a given break is an isolated one or part of a double strand break based on the definition that two breaks on opposite strands within ten base pairs of each other create a double strand break.

Indirect Mechanism

The production of DNA damage by water radicals is called the indirect mechanism. In the radiolysis of water, four major chemical species, ·H, e^-_{aq}, ·OH, H_3O^+, are produced during the first picosecond with yields of 0.88, 5.00, 5.88, and 5.00 respectively. These species undergo diffusive motion in the system and their individual fates are determined by: (1) reactions with each other to produce molecular products, (2) reactions with scavengers, and (3) reactions with sugars or bases. Of the radicals which interact with the DNA, most of the ·H react with the bases, all of e^-_{aq} react with the bases, 20% of ·OH react with the sugars and the rest (80%) with the bases. H_3O^+ does not react with DNA but it can react with other water radicals. Hence, as far as strand breaks are concerned, our focus has been on ·OH. Our procedure has been to determine the decay curves for ·OH as a function of time using a Monte Carlo approach to simulate the diffusion phenomenon. These decay curves have been calculated for pure water and for systems which include scavengers (e.g., tris). For electron tracks as well as heavy particle tracks, these curves have been determined before proceeding to calculate DNA strand breaks.

For each OH radical whose diffusive motion is to be followed, the initial position coordinates (distributed uniformly in space around the DNA) are determined using random numbers. Each radical is followed in space and time by simulating diffusive motion. Corresponding to an interval Δt of "jump" time, the reaction probability P for an OH radical under consideration is determined from the appropriate previously obtained decay curve. Next, a random number between 0 and 1 is selected and if it happens to be smaller than or equal to P, the ·OH is removed from further consideration. If the number is greater than P, the next jump for ·OH is taken. In this manner a surviving OH radical is followed until it is found to be within the reaction radius of a sugar or a base. The respective reaction radii of the various sites have been calculated using Smoluchowski's theory and the rate constants for isolated molecules. The respective radii are: ·OH + sugar, 1.0 Å; ·OH+A, 3.6 Å; ·OH + T, 3.6 Å; ·OH + G, 5.2 Å; and ·OH + C, 3.6 Å. Although the rate constants used for determining the reaction radii are for isolated molecules, in a separate calculation it has been verified that, when the respective sites are present in the DNA structural configuration, there is a reduction in the resultant rate constant per molecule compared to the isolated case by a factor of about ten. Furthermore, it has also been verified that, with the above-mentioned reaction radii, about 20% of all the ·OH that react with DNA are absorbed at the sugar sites and the rest (80%) with the bases. These are experimental facts that must be satisfied by any reasonable theory.

We keep a record of: (a) how many OH radicals are lost from the initial number on their way to a DNA molecule, (b) how many react with the sugar moiety, and (c) how many react with the bases. The total number of trials we take for hydroxyl radicals is typically about 200,000 which yields results reproducible within ± 2%. The use of precalculated decay curves saves a considerable amount of computer time. Based on these procedures, the D_{37} (indirect effect) values for the induction of single strand breaks have been calculated. The D_{37} dose is defined as the dose required to reduce the fraction of the number of undamaged DNA molecules to 37% of the initial (unirradiated) value. Separate calculations have been done for the core and the penumbra, using appropriate decay curves. From the D_{37} values we have computed the indirect effect cross sections for single strand breaks for high-LET particles according to the formula

$$\sigma_{SSB} = KL\left(\frac{f_{core}}{D_{37}^{core}} + \frac{1-f_{core}}{D_{37}^{pen}}\right),$$

where K = 16.02, σ_{SSB} is in $Å^2$, L is the LET in $eV.Å^{-1}$, D_{37} values are in krad and f_{core} is the fraction of energy deposited in the core.

Indirect production of double strand breaks (breaks on opposite strands within 10 base pairs) has been computed by assuming that 2.0% of the single strand breaks (experimental result) also result in breaks on opposite strands. This procedure is adopted in the absence of a satisfactory mechanism for the production of double strand breaks due to indirect effects. Two hydroxyl radicals attacking opposite strands in our calculations produce insignificant double strand break yields (about two orders of magnitude smaller than the data).

Direct Mechanism

The main features of our theoretical model of the direct effect /10/ are summarized in the following. Track structure and stopping power theory in combination with the detailed geometry of DNA molecules are used to calculate the average energy deposition by fast charged particles on DNA. Since the actual physical energy deposition processes are stochastic in nature, we use Poisson statistics to provide a detailed description of the distribution of the resulting states of excitation and ionization of the molecule. The average energy of an excitation (or ionization) on the DNA has been calculated from existing oscillator strength data for biological molecules to be approximately $\langle E \rangle = 30$ eV. Ionization of an atom on the DNA sugar-phosphate backbone is assumed to be followed by one of two different types of biochemical change, deprotonation or direct dissociation, both of which lead to DNA strand breaks. Base damage has been assumed not to lead to the formation of strand breaks.

For convenience and consistency, we have used the same three-dimensional geometric model of DNA for direct effect calculations as used in the indirect effect calculations. The spatial orientation of a given track can be represented by its impact parameter or distance from the DNA central axis, the angle between the track and the DNA axis, and the core radius r_c. The coordinates of a particle track and the DNA model determine which sugar-phosphate molecular groups lie within the core. Restricting ourselves to glancing collisions only and using the Bragg rule in combination with a simplified stopping-power formula, we calculate the average energy E_g lost to a collection of atoms (sugar-phosphate) inside the track core to be

$$E_g = \frac{K}{2\pi r_c^2} \sum_i Z_i \ln (2 mv^2 / I_i) .$$

Here $K = 2\pi z^2 e^4 / mv^2$, where z and v are the charge number and velocity, respectively, of the incident particle and e and m are the electronic charge and mass. Z_i and I_i are the number of electrons and the mean ionization potential, respectively, of the ith atom and the summation is over all atoms in the molecular group.

Since the energy density in the core is a sum of glancing collisions and low-energy-knock-on collisions, we have modified E_g to take into account the δ-rays by writing

$$E_d = E_g [1 + \frac{1}{1+2\ln(r_p/r_c)}]$$

The penumbra of a track has been treated as a collection of independent electrons with an appropriate energy distribution. Each electron track in the penumbra has been treated in an analogous way to that for the heavy particle tracks described above. Calculated values for the core and penumbra have been combined to produce the final results.

The average number of ionizations (and or excitations) on a sugar-phosphate molecule lying within a track core is

$$n = E_d / \langle E \rangle .$$

Using Poisson statistics, the respective probabilities of no ionization, one ionization, two ionizations, etc., are

$$P_i = \frac{n^i e^{-n}}{i!}, \qquad i = 0, 1, 2,$$

The probability of one or more ionizations is given by

$$P_{\geq 1} = 1 - P_o = 1 - e^{-n} .$$

In our calculations, for each incident particle track (or penumbra electron track), we determine the probability of ionization, $P_{\geq 1}$, at each reaction site within the track core radius r_c. We generate an "event" by choosing a random number for each site. If $r\# < P_{\geq 1}$ for a particular site, we consider an ionizing reaction to have occurred with the production of a DNA strand break, either by direct dissociation or deprotonation. Breaks occurring close enough to each other on opposite strands (i.e., within 10 base pairs) lead to double strand breaks. An event is then classified into one of the following categories:

(1) no breaks (NB),
(2) one or more single strand breaks (SSB), and
(3) one or more double strand breaks (DSB).

If we consider N incident particle tracks uniformly distributed over an area A, yielding N_{SSB} single strand breaks and N_{DSB} double strand breaks on DNA of molecular weight M, the corresponding normalized cross sections are given by

$$\sigma_{SSB} = N_{SSB}/(MN/A) ,$$
$$\sigma_{DSB} = N_{DSB}/(MN/A) .$$

It is frequently more convenient to discuss strand break production in terms of yields of breaks per unit radiation dose rather than cross sections. The yields, Y_x, corresponding to the normalized cross sections are given by the following expressions:

$$Y_{SSB} = \sigma_{SSB}\rho / LET ,$$
$$Y_{DSB} = \sigma_{DSB}\rho / LET ,$$

where ρ is the mass density of the medium and LET is the total linear energy transfer of the incident particle. In terms of conventional units of $(Å)^2$ for σ, keV/µm for LET and rad for dose, the expression for the strand break yield per rad per dalton becomes

$$Y = 6.2 \times 10^{-10}\, \sigma / LET .$$

In the expression for Y, it should be kept in mind that σ depends on the track structure.

RESULTS

Cross-Sections

Results of our calculated strand-break cross-sections for representative particles, He and Ne, are shown in Figure 1. Single strand break cross-sections vary approximately linearly with LET and the corresponding dependence for double strand scissions is LET^2. For single strand breaks, it appears that both the indirect mechanism (evaluated for scavenger concentrations chosen to simulate cellular conditions) and the direct mechanism have roughly equal cross-sections which introduces a possible new phenomenon. Namely, one strand can be broken by the indirect mechanism and the other by direct mechanism. This effect has not been included in the present model. Below 30 keV/µm, radical mechanisms seem to create double strand breaks with a greater efficiency than the direct ionization events. Above this LET value, the reverse is true.

Negligible Effects of Tris Radicals

In Figure 2, we have plotted the variation of the D_{37} values as a function of scavenger (tris) concentration for ^{60}Co-γ radiation. When the molarity is low, the average ·OH migration distance is very large which results in dominance of the indirect action. The dotted line in Figure 2 is the result of indirect action only and the solid line represents the sum of indirect and direct actions according to the relationship:

$$1/D_{37}^{total} = 1/D_{37}^{indirect} + 1/D_{37}^{direct}$$

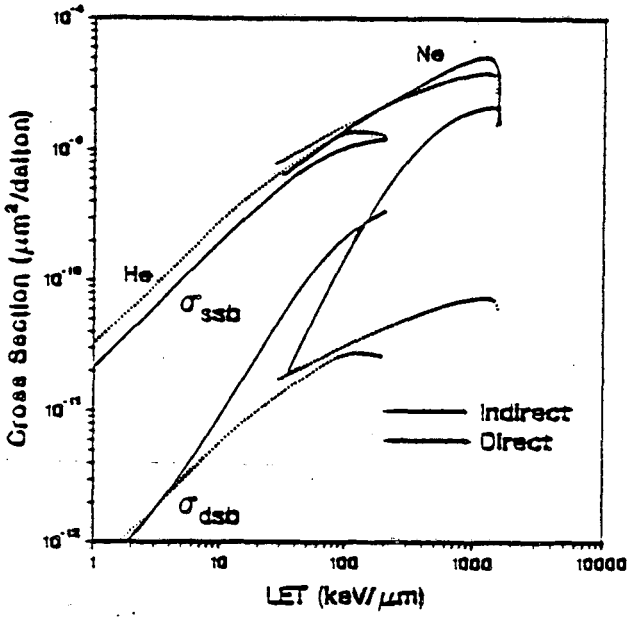

Figure 1. Calculated cross-sections for strand break formation by direct and indirect effects for He and Ne as functions of LET. The total cross-section is the sum of the two contributions. At the lowest LET's, the indirect effect cross-sections are larger and at the highest LET's, the direct effect dominates.

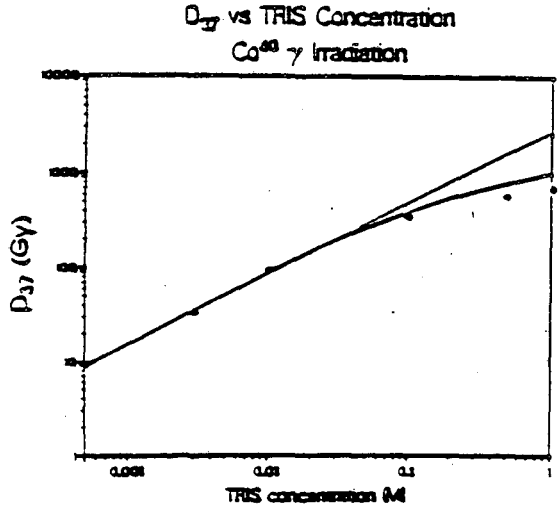

Figure 2. Variation of D_{37} for single strand breaks as a function of tris-HCl concentration. The dotted line represents calculated results for indirect effects only, while the solid curve includes direct and indirect effects. Solid circles are data points from experiments of Ruth Roots, Ernst Henle, and Conrad Trumbore (private communications).

For low concentrations of tris, there is no difference between $1/D_{37}^{total}$ and $1/D_{37}^{indirect}$ indicating that the contribution due to direct action is negligible. This feature is expected because -OH can migrate from large distances and direct excitation and ionization of DNA has a much smaller probability than reactions with water molecules which are present at 55 M. However, as the tris concentration becomes larger than 0.04 M, the $1/D_{37}^{direct}$ values start influencing the $1/D_{37}^{total}$ values, i.e., the direct action becomes increasing important. These results are supported by the experimental data as indicated by the solid circles /13/. Since our calculation does not take into account effects of tris-radicals (mainly formed from the reactions of tris with -OH), the agreement between theory and experiment suggests that these species do not cause appreciable numbers of strand breaks.

It is important to note that at 0.5 M tris, the direct effect is appreciable. At this molarity the average ·OH migration distance is about 3 nm, which is similar to what it is believed to be in a cellular complex. The calculated D_{37} value for single strand breaks at this concentration is 749 Gray and a value of 700 Gray has been measured experimentally.

One of the aims of this work is to compare our calculations with strand break data when cells are irradiated with various qualities of radiation. In Figure 3, such a comparison has been made by using data from reported measurements of several investigators. In these measurements, the experimental conditions were *manipulated* so that no (or minimal) enzymatic repair of strand breaks was allowed. It can be seen from the comparison that the theoretical and experimental results are qualitatively (and even quantitatively) quite similar, providing some confidence in the calculational procedure described in this paper.

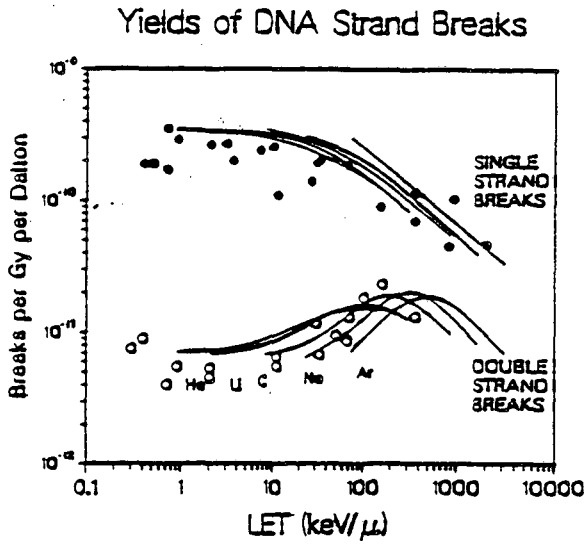

Figure 3. The smooth curves are calculated yields (including both direct and indirect effects) of single and double strand breaks plotted *vs.* LET. For comparison, a selection of experimental measurements from the literature of radiation-induced initial (enzymatic repair inhibited) strand break yields for a variety of mammalian cell types is also plotted.

Clusters of DNA Damage

We have used our present model to estimate quantitatively the extent of sugar and base damage at or near the sites of radiation-induced single and double strand breaks. Figures 4a, 4b, and 4c represent schematically the distribution of DNA damage for typical events. Sugar damage resulting in a strand scission is indicted by a missing "S", and base damage is indicated by "B*". Figure 4a shows typical low-LET events, characterized by isolated single strand breaks and damaged bases with an occasional double strand break. Figures 4b and 4c show typical double strand break events for 10 MeV/n neon (450 keV/μm). These events are characterized by a substantial number of damaged sites (sugars and bases) spread over a region of 10-15 base pairs.

These detailed pictures of strand breaks can be summarized by curves which show the average number of strand scissions and the average number of damaged bases for each single or double strand break. Results of these calculations for helium and neon irradiation *vs.* particle LET are displayed in Figures 5 and 6 respectively. At low LET, as one would expect, the number of strand scissions per single and double strand break remains very close to one and two respectively, and the probability of associated base damage is small. For helium at high LET (~ 100 keV/μm) one extra strand scission and one damaged base, on the average, accompany each double strand break. For incident neon ions at high LET up to seven total breaks (five extra) and up to five damaged bases are associated with each double strand

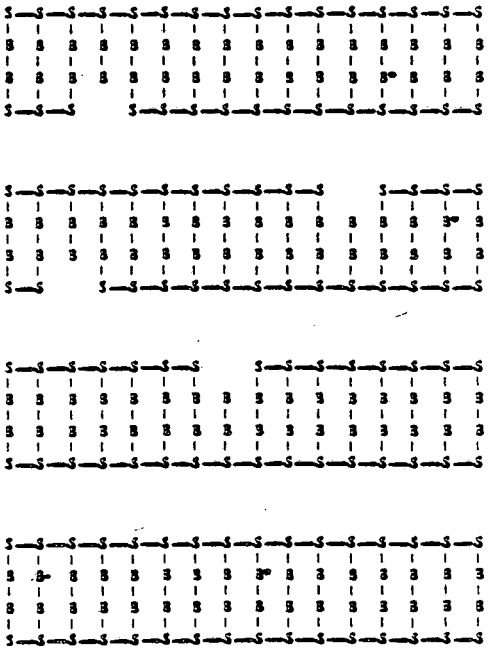

Figure 4a. Diagrams of typical low-LET strand break events. Second event from top is a double strand break event.

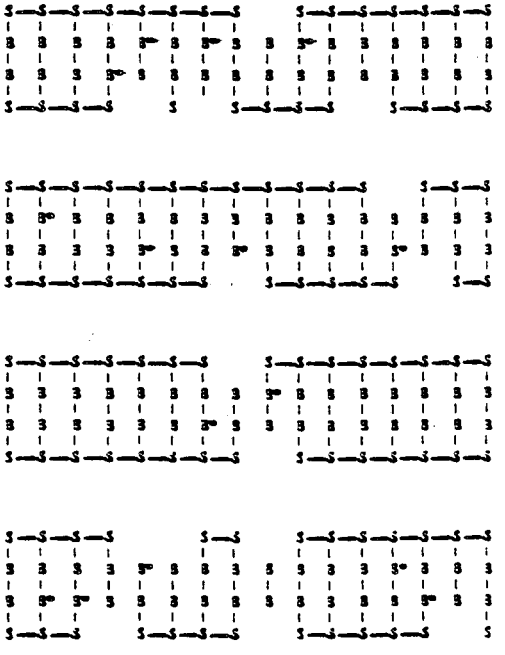

Figure 4b. Diagrams of typical high-LET (450 keV/μm) double strand break events.

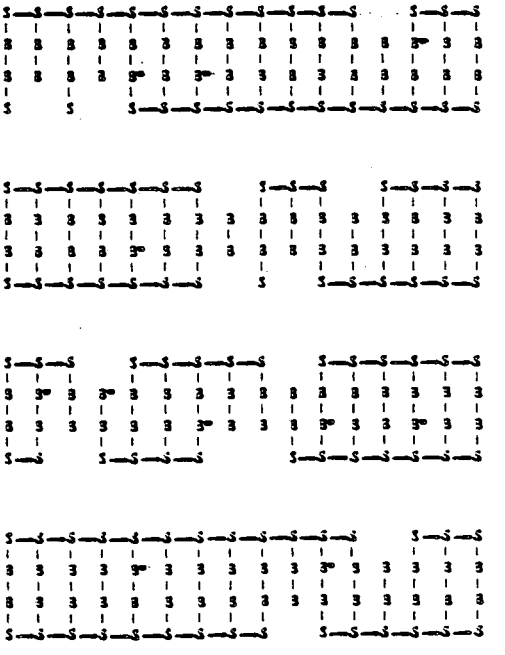

Figure 4c. Diagrams of additional high-LET (450 keV/μm) double strand break events.

Strand scissions (damaged sugars) are indicated by a missing "S" and damaged bases by "B*".

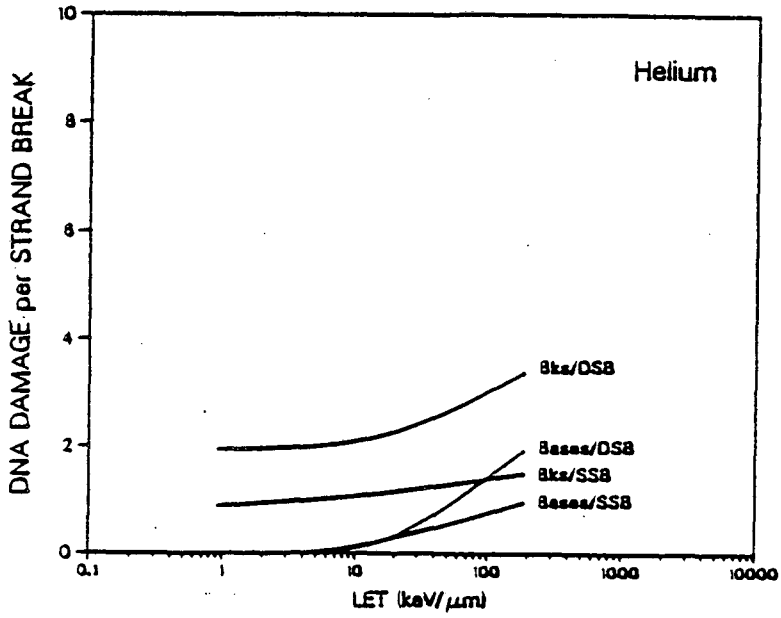

Figure 5. Average number of strand scissions and damaged bases per single and double strand break for incident helium ions.

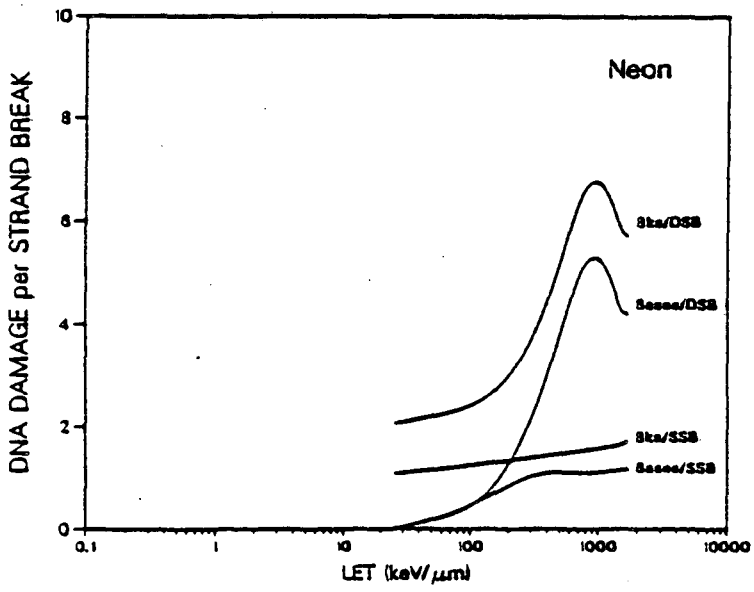

Figure 6. Average number of strand scissions and damaged bases per single and double strand break for incident neon ions.

break. On the other hand, total scissions per single strand break remain relatively small (< 2) even at the highest LET since events with large numbers of strand scissions almost always satisfy the criteria for double strand breaks.

The results of these calculations indicate that there is a qualitative difference in the nature of double strand breaks induced by low-LET and by high-LET radiation. Most lesions produced by low-LET radiation are simple, isolated, single (and sometimes double) strand breaks. On the other hand, double

strand breaks produced by high-LET radiation almost always are associated with multiple lesions either on adjacent sugars or bases or frequently both.

DISCUSSION

A theoretical model has been presented in this paper which predicts the yields of single and double strand breaks in a DNA molecule induced by different qualities of radiation. The model correlates track structure of energy deposition with chemical and biochemical changes which ultimately result in the formation of observable strand breaks. Although there are parameters involved in the calculations outlined above, they have not been adjusted to match the experimental data. For example, 17 eV has been taken as the energy needed to create a radical pair such as ·H and·OH. This number is not known very well for liquid water and hence should be considered as a parameter without a strong fundamental basis. However, this same value has been used in our previous models in the analysis of the Fricke dosimeter system (an aqueous solution of ferrous sulphate) and we believe that this number should not be arbitrarily varied to fit experimental data for another aqueous system. Since in the present context we have been dealing with DNA in aqueous solution, we have used 17 eV as the energy required to create a radical pair. Most other parameters used are well known diffusion constants, rate constants, base pair separation distance, etc.

In order to avoid the complexities of cellular media, a very simple system consisting of linear DNA molecules in water (with scavengers) has been used for modeling purposes. This is an oversimplification of the actual biological case. However, when compared with experimental data from exposures of mammalian cells, the results from the proposed theoretical model seem to be in fairly good agreement. Hence, it appears that the model has some validity with respect to the production of initial damage.

In spite of this success, we believe that there are several drawbacks to the model in its present form which need improvement. For example, energy migration along the DNA chain is a well known phenomenon which suggests that the site of damage may be quite different from the location where energy was deposited. What effect this energy migration has on strand break formation is not clear at the present time. Similarly, no attempt has been made in the model discussed here to understand the effects of structured water associated with DNA molecules. It is possible that structured water behaves differently than bulk water. It is, therefore, important to understand how a hydroxyl radical propagates through the bound water before it gets to a particular DNA site (sugar or base).

Another weakness in the present model is the lack of a suitable mechanism for the production of double strand breaks by hydroxyl radicals. As discussed previously, in our model, reactions by two or more hydroxyl radicals from the same incident track do not yield an adequate number of double strand breaks. It is possible that a single ·OH may be responsible for the production of these types of alterations in a DNA molecule. If this is so, one needs to understand the mechanisms involved before they can be incorporated in a model.

In the present model no account has been taken of the creation of double strand breaks by cooperative phenomena in which one strand is broken by the indirect effect and the other strand by the direct effect. We do not expect this mechanism to introduce a large contribution to the overall yield of double strand breaks; nevertheless, inclusion of this effect should improve the accuracy of the results presented in this report.

From these and other considerations, it is clear that a mechanistic model such as the one presented here needs further improvements, but even in its present state the results seem to provide clear evidence that high-LET double strand breaks may be qualitatively very different from most low-LET breaks. We are particularly interested in the potential effect that these differences may have on double strand break repairability and on biological end points such as chromosomal aberrations, cell death, mutations, and neoplastic transformation. We are currently exploring ways to incorporate these results into models for cell transformation and mutation.

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