

Mechanisms for the Biological Effectiveness of High-LET Radiations

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Radiations of high linear energy transfer (LET) have long been known to have greater biological effectiveness per unit dose than those of low LET, for a wide variety of biological effects. However, values of relative biological effectiveness depend considerably on the biological system and in some instances the values are clearly below unity. The differences between high- and low-LET radiations may be due to many factors, almost all of which are related to radiation track structure in one way or another, and some can in principle lead to qualitative as well as quantitative differences between the radiations. Explanations for LET-dependent differences in effectiveness are discussed over a variety of levels from the multicellular and cellular scale down to the DNA scale, with illustrations from radiobiological data. Information from well-defined slow light ions provide particularly useful analytic data, but practical issues extend also to neutrons and fast heavy ions, which may compound high- and low-LET features. It is suggested that effectiveness of the radiation is determined predominantly by the complex clustered damage that it produces in DNA, but that for high-LET radiations long-term effects are in some instances limited by single-track-survival probabilities of the traversed cells.

INTRODUCTION

Already early in the 20th century it was demonstrated that densely ionizing (high linear-energy-transfer, LET) radiations, such as neutrons and α -particles, could have a greater biological effectiveness than sparsely ionizing (low-LET) X-rays or γ -rays, in killing of fern and bacterial spores for example. The reverse was found in other biological systems, such as sex-linked lethal mutations in *Drosophila*. For human cells, the relative biological effectiveness (RBE) of high-LET radiations has direct practical implications in therapy applications and in assessing risks from environmental and occupational exposures, and it also provides analytic information on the underlying mechanisms of radiation biology.

For many relevant effects in mammalian systems there is a general tendency to increasing RBE with increasing LET, up to a maximum (at ~ 100 – 200 keV μm^{-1} for α -particles) followed by a decrease at very high LET. However, the numerical values of RBE for a given LET can vary by

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large amounts (even orders of magnitude) depending on other physical and biological conditions. Values of less than unity up to a few hundred have been deduced from experimental data. Common general tendencies in mammalian systems are¹⁾: for mutation RBEs to be greater than cell inactivation RBEs; for lighter ions (such as protons, or α -particles) to reach their peak at lower LETs than faster ions (such as α -particles, or carbon-ions, respectively); for RBEs to be larger at lower doses and dose-rates; and for radiosensitive cells to show lower RBEs than radioresistant cells. There are, however, many exceptions to such generalities. Therefore, we should conclude that there are a number of competing mechanisms and diverse factors that determine the effectiveness of high-LET radiations. This makes it difficult to identify the individual mechanisms and to predict RBEs for individual practical application.

Expressing the relative effectiveness in terms of ratios of particle fluence rather than of absorbed dose would not resolve the problems of biological diversity. On the contrary it would greatly expand the overall range of effectiveness values²⁾, increase the range of variability and raise fundamental and practical problems in specification and measurement of fluence of reference radiations, neutral particles and mixed fields.

The problem is compounded by observations that high-LET effects can sometimes be qualitatively as well as quantitatively different from low-LET. In these cases the attempted use of an effectiveness ratio may be unhelpful, RBE may implicitly tend to infinity and there may be no low-LET data for meaningful comparison. Reported examples of such differences include³⁾ induction of sister chromatid exchanges in G_0 -irradiated human lymphocytes, induced chromosomal instability in haemopoietic stem cells⁴⁾, the spectrum of chromosome aberrations in human fibroblasts, slower cellular recovery in tissues and persistence of changes in mammary epithelial cells in vivo. These may be related to the demonstrated lower repairability and persistence of DNA damage from high-LET radiations or to other, as yet unidentified, consequences of the spatial and temporal pattern of subcellular damage.

Despite these variabilities in effectiveness, essentially all the biological differences between high- and low-LET radiations must arise from the track structures of the ionizing charged particles that are set in motion in the biological cells. The microscopic spatial distributions of ionizations (and excitations) are believed to be of greatest importance, but temporal aspects may also be relevant. To date no high-LET-specific atomic or nuclear processes have been implicated; even the recent K-shell-ionization hypothesis of Chetioui⁵⁾ is suggested to act mainly via clustering of ionizations in the local track structure.

In seeking the dominant mechanisms of high-LET effectiveness, there are distinct advantages in using slow light ions for experimental and theoretical analyses, because their tracks are best defined, both in experimental studies and in theoretical track structure simulations. Much of the analysis and discussion, therefore, concentrates on slow α -particles, of for example 3.3 MeV ($120 \text{ keV}\mu\text{m}^{-1}$), which are of near maximum RBE for many cell effects. With such particles, cell monolayers can be irradiated in the 'track segment' mode from external radionuclide sources or accelerators to optimize the homogeneity and systematic control of the LET of the tracks in the cells. Systematic investigations of this type with slow protons have shown that the RBEs of protons for a variety of cellular effects are greater than those of α -particles of the same LET. The high-LET mechanisms that are identified from such studies should be applicable also to other

less tractable high-LET radiations. Direct mechanistic analysis of experimental data for neutrons or fast heavy ions are confounded by a variety of complications. For example, from fission neutrons the cell receives a very wide variety of tracks, mostly of recoil protons of energies about 0–10 MeV, LETs $\sim 4\text{--}90\text{ keV}\mu\text{m}^{-1}$ and ranges about 0–1200 μm , including some tracks which stop, start, cross or are entirely internal to a cell nucleus. Furthermore, there is always a dose-component from accompanying low-LET γ -rays, amounting for example to 33% from an unshielded ^{252}Cf fission source. Interpretation of effects from fast heavy ions is complicated by their long delta-ray electrons, which may travel across many cells (even millimetre distances) and irradiate them with essentially low-LET electrons. Mechanistic understanding of effects of neutrons should be aided by synthesis of understanding of the mechanisms of action of the charged particles in the recoil spectrum.

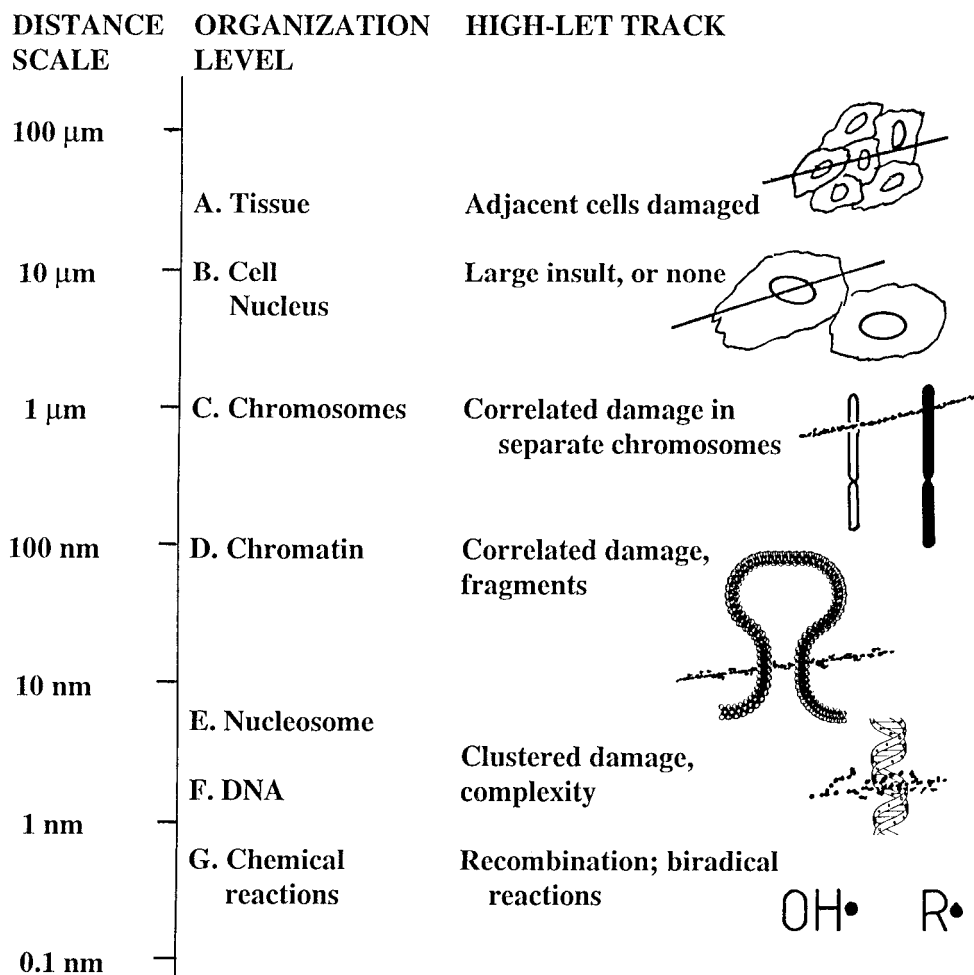
DISTANCE-SCALE FOR HIGH-LET MECHANISMS

It is not surprising that a high-LET track is more likely to damage a cell than a single low-LET track. However, on the basis of equal absorbed dose (approximately equal average numbers of ionizations per unit volume), there are two competing trends. The small numbers of high-LET tracks per unit dose (approximately proportional to $1/\text{LET}$) are less likely to pass through any given target or microscopic region of the cell, but if they do they are more likely to cause substantial damage. On this basis alone it is not obvious *a priori* whether 1 α -particle, say, would be more biologically damaging to the DNA of a cell than would several hundred electrons, for a similar dose to the cell nucleus.

When the α -particle is found experimentally to be the more biologically effective, as is usually the case, the explanation must lie in the spatial (and/or possibly temporal) correlations within its single track, but over what distances and levels of biological organisation? Figure 1 illustrates levels of organisations from multicellular tissue down to individual small molecules and atoms, covering a scale of about six orders of magnitude. High-LET-track correlations are apparent at all of these levels, which will be briefly discussed below.

ATOMIC LEVEL

Starting first at the most microscopic level (G, in Fig 1) of individual chemical reactions of radiation ionized molecules (free radicals), radiolysis experiments have shown clearly that the yield of single radicals (e.g., OH) and their reaction products decrease with increasing LET, largely due to recombination and reaction of radicals within the same track. The yields of biradical products (e.g., H_2 or H_2O_2) therefore increase, but only modestly. These are clearly insufficient to explain the observed RBEs of high-LET radiations, although they may be a pointer to the biological relevance of clustering of ionizations¹. The chemical trends are mirrored by a reducing yield of simple DNA damage (such as single-strand breaks) with increasing LET, and by little LET dependence in the yields of simple bi-radical damage such as double-strand breaks (Fig. 2).



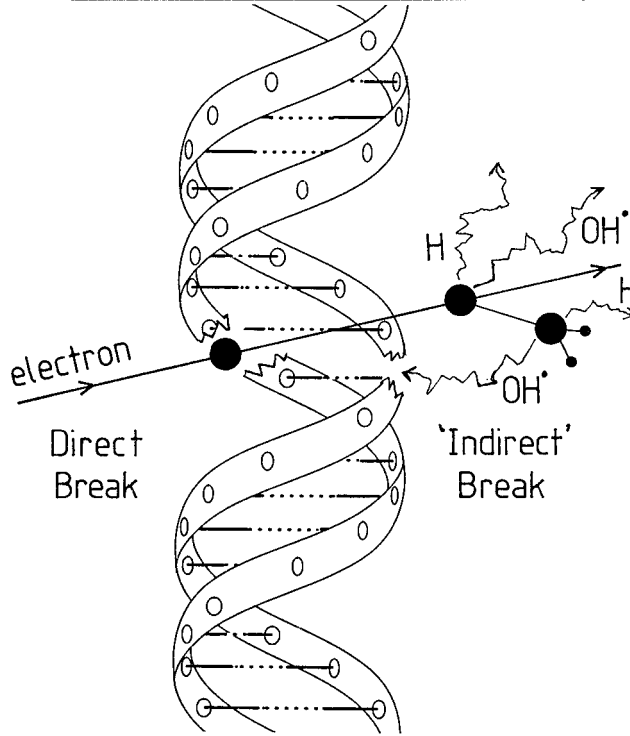
**High-LET and low-LET radiations are different at all these levels.
Which level(s) dominate the biological effectiveness?**

Fig. 1. Dimensions and levels of biological organisation over which high-LET radiation track structure may be important in determining biological effectiveness.

TISSUE LEVEL

Now consider the large dimensions of tissues (A in Fig. 1). It is conceivable that expression of cellular damage may be enhanced by death of, or substantial damage to, adjacent cell(s). At low doses (ie single tracks in a cell) such a correlated effect would occur almost exclusively with

A small 'clustered damage' (simple dsb)
resulting from a local cluster of
ionizations within a single track:



Radical diffusion distances in cells
are very small (< 4 nanometres).

Fig. 2. Illustration of direct and indirect action of a radiation track is causing DNA damage, in this case a double-strand break by combined action of the two modes from a small cluster of ionizations in the track.

high-LET radiations only. It is on such a basis, including transfer of DNA from the disintegrating cell, that Mole and van Bekkum each hypothesised a very high relative effectiveness for neutron-induction of leukaemia at low doses, relative to a vanishingly small effectiveness of low dose γ -rays. This mechanism has not been demonstrated, although experiments with epidermal neutrons could in principle provide a test of expectations⁶⁾.

CELLULAR LEVEL: SINGLE-TRACK SURVIVAL

An important factor in determining RBEs for viable effects does arise at the level (B) of the cell (or its nucleus) (B in Fig 1). First, we can probably exclude that long-distance correlated damage in the cell nucleus is a necessary condition for high-LET effects. This is the implication of the observed high-LET-like cellular responses to ^{125}I decays in DNA and of the observed high effectiveness for cell killing, and induction of chromosome aberrations, of 24 keV epithermal neutrons⁷. The recoil protons have mean range of $< 0.3\mu\text{m}$ (maximum $< 0.5\mu\text{m}$) but apparently this does not limit their effectiveness as high-LET radiation (LETs $\sim 10\text{--}70\text{ keV}\mu\text{m}^{-1}$). The dosimetry used in these and similar experiments with the 24 keV neutrons is not subject to questions about the calibration for ionization chamber dosimetry at these energies, because alternative dosimetry methods were applied⁸.

However, there is an important consequence of correlations on the scale of the cell nucleus. For most high-LET radiations, with track lengths greater than cell dimensions, the probability of a cell being killed by a single track can severely limit the potential for expression of viable effects such as mutation, as well as transmissible aberrations, oncogenic transformation and cancer initiation. Due to correlation of potentially mutagenic damage and lethal damage along the *same* single track through a cell, the mutagenic damage cannot be expressed. This factor must be accounted for in the experimental data before the systematics and mechanisms of the underlying mutagenic process can be fully analysed. It is not accounted for by the usual expression of mutation frequencies as 'per survivor', because this takes into account only uncorrelated cell killing by other tracks. Unfortunately many of the available data on long-term effects such as mutation and cancer are not accompanied by information from which this correlated-survival factor can be deduced. However, it is clear that the single-track survival probability (S_1) can vary from $> 90\%$ down to essentially zero, depending very strongly on cell type and also on cell geometry and radiation type.

Table 1 lists values of S_1 for a $120\text{ keV}\mu\text{m}^{-1}$ α -particle traversing a variety of cell types, derived from measurements of dose-survival curves and cell-nuclear areas (by confocal microscopy⁹), obtained at the MRC Unit. It is seen that even for these optimally-effective α -particles, some cell types have about 90% probability of surviving the track and therefore very little reduction in their ability to express mutagenic damage. At the other extreme normal murine bone marrow pre-B cells have no detectable ability to ever survive such a track through any part the cell¹⁰, and this must radically reduce to essentially zero their expression of genetic damage. The available data suggest a tendency for the haemopoietic cells to have lower S_1 -values than fibroblastic cells. Data are not available for many cell types, including stem cells in various tissues that might be of particular interest in assessing long-term mutagenic and carcinogenic risks.

Even if the underlying (intrinsic) mutagenic mechanisms and their radiation dependence were identical in all these cells, it should be expected that the RBEs for mutagenesis for a given radiation would vary by very large factors, even two orders of magnitude, simply on the basis of this single-track correlated killing of cells. Consequently there should be every expectation that a

Table 1. Single-track-survival probabilities (S_1) for 120 keV μm^{-1} α -particles

| Cell Type | Do (Gy) | Mean Nuclear Area (μm^2) | S_1^* |
|---|-----------|---------------------------------------|---------------------------------|
| Fibroblasts: | | | |
| Human, primary: HF12 | 0.40 | 164 | 0.70 |
| HF19 | 0.34 | 155 | 0.62 |
| Hamster V79 | 0.83 | 130 | 0.82 |
| Mouse 10T1/2 | 0.61 | 201 | 0.83 |
| Keratinocytes: | | | |
| Human, HPV transformed | 0.57 | 134 | 0.75 |
| Epithelial: human thyroid SV40 transformed | ~0.5 | 293 | ~0.9 |
| Lymphocytes: | | | |
| Human, primary | 0.74 | 40 (cell) ~24–30 (nucleus) | 0.34 (cell) ~0–0.1 (nucleus) |
| Haemopoietic (mouse): | | | |
| Stem cells CFU-S (CBA) | 0.55–0.61 | ~40 (cell) | ~0.08–0.18 (cell) |
| “ “ “ (BDF ₁) | 0.61–0.69 | ~40 (cell) | ~0.17–0.27 (cell) |
| Multipotential LyD.9 line | 0.90 | 52 | 0.59 |
| Multimyeloid B6SütA line | 0.67 | 44 | 0.34 |
| Pre-B, <i>v-abl</i> transformed | 0.68 | 46 | 0.38 |
| Pre-B, primary (Balb/c) | 0.39 | 41 (cell) | 0 (cell) |
| Mature B, primary (Balb/c) | 1.16 | 32 | 0.48 |

* Probabilities (S_1) for a cell to survive after the passage of a single track through its nucleus, evaluated as $1 - 0.16L/(AD_0)$, where L is LET in $\text{keV}\mu\text{m}^{-1}$, D_0 in Gy is the reciprocal slope of \ln -survival versus dose curve and A is projected area of cell nucleus in μm^2 ¹¹. In some cases the cell area has been used to give probability of survival after random passage through any part of the cell.

single specified quality factor (Q) or radiation weighting factor (W_R) for a given radiation, as used in radiological protection, would be a poor representation of the actual RBEs for long-term effects in specific tissues.

Earlier simple analyses have suggested that the observed RBE-LET relationship for *hprt* mutations in human fibroblasts may be distorted by a factor of about 2 for slow ions at the higher LETs as a result of this correlated killing¹¹, but much greater distortions are expected for other cell types. The impact of the single-track survival limitation is more difficult to evaluate for neutrons, with the variety of recoil particle types, energies, LETs and ranges, although it could be synthesized from systematic information on monoenergetic particles and monoenergetic neutrons if available including for tracks shorter than the cell size. On the bases of the existing data it is expected that S_1 factors for protons would vary from essentially zero to 100% depending on the cell type and the proton energy. Fast ions present additional problems due to their large track width with long-ranged delta rays.

Particularly useful information on intrinsic high-LET mutability of cell types, may be obtainable with epithermal neutrons because the short track lengths will cause the probability S_1 to be ≈ 1 and hence cause negligible reduction from the intrinsic mutagenic response. From

unpublished data on survival of murine haemopoietic stem cells exposed to 24 keV neutrons it can be estimated that for these cells $S_1 \approx 95\%$. However, the author is unaware of any mutation measurements with these neutrons. High-LET short-track radiations may represent a larger mutagenic risk in sensitive cell types than would conventionally be inferred from other high-LET radiations.

CHROMOSOME-DAMAGE CORRELATIONS

A particularly long-standing assumption of radiation cytogenetics has been that high-LET effectiveness is largely due to single-track correlation of damage in two separate chromosomes at distances of $\sim \mu\text{m}$ apart, initiating chromosome exchange aberrations between the two break points. However the assumption may well be incorrect in the light of accumulating information since the 1970's with short-track irradiations (ultrasoft X-rays and epithermal neutrons) and recently enhanced by chromosome fluorescence in situ hybridization (FISH) techniques. 24 keV neutrons have been reported to be at least as effective as fission neutrons in inducing exchange aberrations^{7,12}; therefore any single-track two-break (hypothesized) exchanges would need to have arisen within less than $0.3 - 0.5 \mu\text{m}$. Much more dramatically, however, C_K ultrasoft X-ray irradiations, producing electron tracks of only $\sim 7 \text{ nm}$, not much greater than the diameter of a single DNA duplex, had previously suggested strongly that an exchange could arise from a *single* radiation-damaged chromosome exchanging with an *undamaged* chromosome. FISH methods have now strongly reinforced this conclusion, showing convincingly that simple exchanges between two chromosomes arise from a single point of damage and therefore with linear dose dependency¹³. These studies revealed, too, that complex chromosome exchanges arise largely from multiple radiation breaks.

Applying these newer hypotheses to high-LET radiations, one might expect that the single-track correlations of damage in separate chromosomes do not confer any strong advantage to high-LET radiations in the yield of simple aberrations (except in so far as the local clustered DNA damage (see below) may be more prone to misrepair), but the correlations do enhance the proportion of complex aberrations. This is consistent with experimental results, which show that about 40% of aberrations from α -particles are complex, even at low doses, whereas they are negligible at low doses of low-LET radiation¹³. Long-term biological consequences of these aberrations are likely to be reduced because most of the complexes are non-transmissible (lethal to the cell). There was observed, however, a substantial proportion of complex insertions after the α -particles and these may be a persisting consequence with a particularly high RBE at low doses. Here again, epithermal neutrons might offer useful new insights into the range of complex-aberration formation, and also on the local ionization density requirements (because these proton recoils tracks are beyond the Bragg peak and so the track are particularly narrow).

CHROMATIN-STRUCTURE LEVEL

A high-LET particle has a clear ability to break a DNA molecule in a number of places as the track passes through interphase chromatin of 30 nm fibre and larger loop structures. It has been hypothesized that this may lead to an excess of DNA fragments over a wide size range from ~ 0.1 kbase to several M base. There is experimental and theoretical evidence of fragments when the cellular DNA is removed for analysis immediately after irradiation¹⁴). However, since the majority of breaks are normally repaired in a cell, it remains unclear whether such long-distance associations of damage on the DNA will reduce their reparability, or will result in persisting fragments due to both ends remaining unrepaired.

LEVEL OF DNA AND NUCLEOSOMES: PRIME IMPORTANCE

This is the level at which the properties of ionizing radiation are likely to be most important in determining biological effectiveness. DNA damage is formed by the ionizations (and excitations) of a track directly in DNA or in surrounding material, mostly water within a radical-diffusion distance of 4 nm from the DNA in the cellular environment. In these ways all ionizing

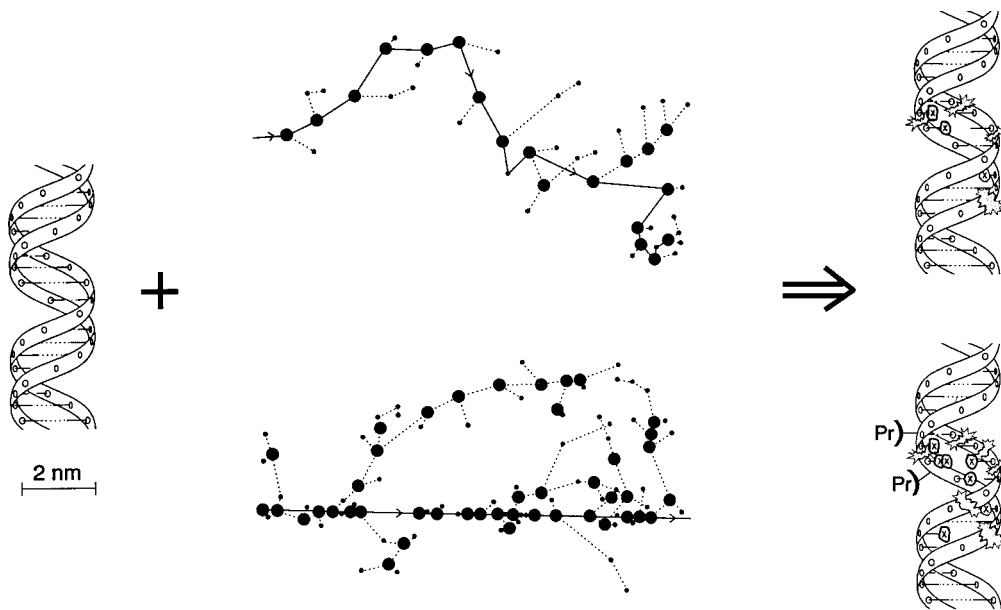


Fig. 3. Illustration of formation of complex clustered damage in DNA by direct and indirect action of radiation tracks which pass through or very close to the DNA. These short segments of Monte-Carlo simulated tracks are drawn on the same scale as the DNA and are from a low-energy electron (upper centre) and a slow α -particle (lower centre). Large dots represent ionizations and small dots excitations. (Reproduced from Goodhead, *Int. J. Radiat. Biol.* (1995) 65: 7-17).

radiations produce single-base damage and single-strand breaks, higher-LET radiations being somewhat less effective because fewer radicals escape intra-track reactions to reach the DNA. It is clear that even low-LET radiations produce also clustered damage at the DNA level due to the closely-spaced clusters of ionizations from the abundant low-energy secondary electrons of say $\sim 0.1\text{--}1$ keV. Thus DNA double-strand breaks (dsb) can result from pairs of direct ionizations or pairs of nearby hydroxyl radicals or by combined direct and OH action (Fig. 2). However, track structure simulations, supported by more recent experimental studies, now show convincingly that a substantial proportion of these low-LET dsb have additional complexity due to further damage by the ionization cluster (Fig. 3). Detailed track-structure simulations indicate that about 30% of 'dsb' from low-energy electrons contain more than two strand breaks¹⁵⁾ and that the complexity is often further enhanced by damaged bases.

Simulations of proton and α -particle tracks show clearly not only that there should be a greater proportion of complex dsb but also greater degrees of complexity (Fig. 3). The overall yields (per unit dose) of 'dsb' of all types show little dependence on LET because of the balance between numbers of particles (inverse to LET) and the increased local ionization density (increasing with LET). However, the proportions of complex dsb increase strongly with LET and the spectrum is shifted towards greater complexity. For the higher-LET α -particles more than 70% of the dsb are estimated to contain more than two breaks and often considerably more¹⁶⁾. Base damage should add substantially to the complexity. The simulations show further that protons produce a more complex spectrum than do α -particles of the same LET. The results suggest also that some of the most complex forms of clustered damage may be unique to high-LET radiations, but they occur at relatively low frequencies so their biological significance is difficult to judge.

Complex clustered damage to DNA shows strong LET dependence that makes it a good candidate as a prime determinant of RBE. It has been hypothesized that the more complex components of the initial spectrum of DNA damage are less readily repaired by the cell and hence are more biologically severe and dominate the final effects (Fig. 4). Consistent with this are experimental observations of slower repair, and a greater unrepaired residue, of DNA 'dsb' from high-LET radiations¹⁷⁾. Earlier biophysical analyses have suggested that high-LET effects are dominated by the damage from large ionization clusters of typically ~ 400 eV (~ 15 ionizations) within distances of $5\text{--}10$ nm, which may represent a nucleosome structure or simply include involvement of a variety of molecules around the DNA³⁾. It is interesting, too, that this suggested approximate microscopic fingerprint of high-LET effectiveness, yields similar LET-dependence of 'ion-kill' cross sections as does the quantitatively successful phenomenological amorphous-track model of Katz¹⁸⁾, including even the 'hook' patterns at very high LET of HZE particles¹⁹⁾. Following the above hypothesis (of complex damage, reduced repairability and consequent biological severity), it should be expected that RBEs for different cell types might depend strongly on their own repair capabilities especially in respect of the less complex, but more abundant, damage. Thus a shift in the repair function of Fig. 4 could substantially alter the spectrum of residual yields and hence the RBE, particularly by altering the low-LET response. This approach has been used in the analyses of DNA-repair kinetics of normal and ataxia-telangiectasia human fibroblasts after γ -ray and α -particle irradiation²⁰⁾.

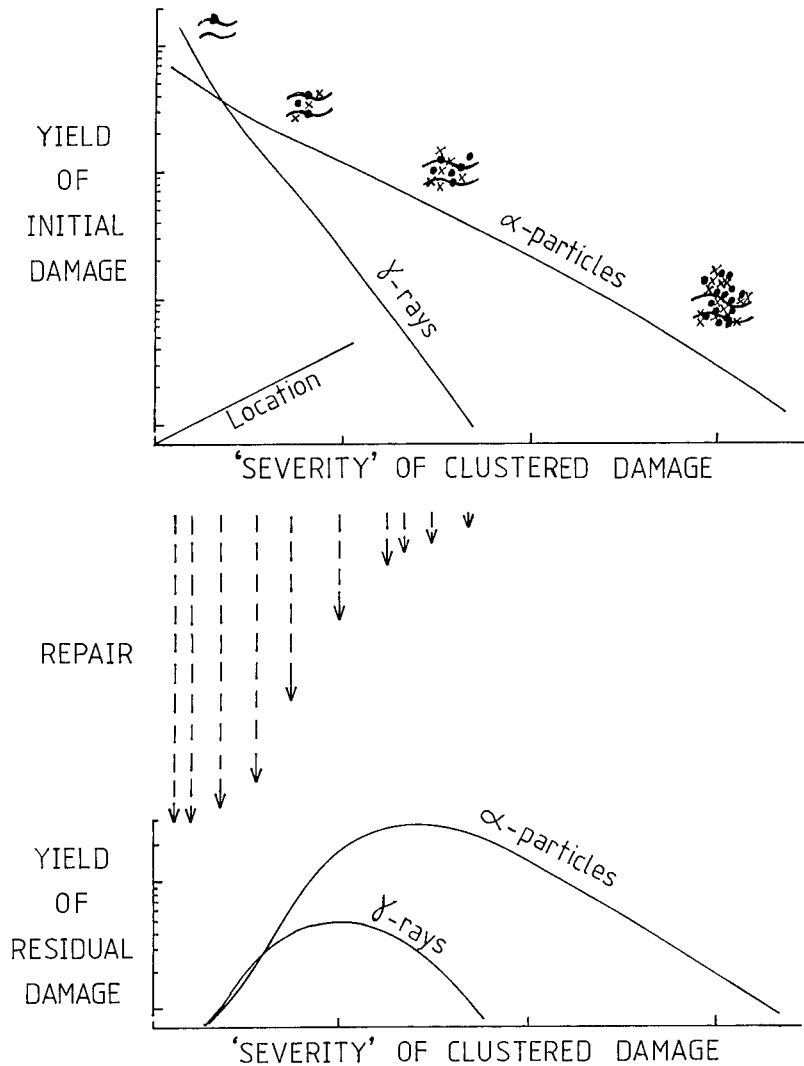


Fig. 4. Hypothesis for raised biological effectiveness of high-LET radiations due to production of complex DNA damage of lower cellular repairability and hence greater biological severity. As illustrated, the high-LET radiation (α -particles) has a much greater biological effectiveness (than the low-LET γ -rays) despite the α -particles producing a smaller total amount of initial DNA damage (such as single-strand breaks) and a comparable amount of initial damage of minor complexity (such as simple dsb). (Reproduced from Goodhead et al., *Int. J. Radiat. Biol.* (1993) 63: 543–556).

TARGETED AND UNTARGETED EFFECTS

All the above discussion has been for effects which are likely to arise from targeted (cis-acting) mechanisms of molecular damage by the radiation track to specific target molecules,

particularly DNA, whether by direct ionization or local free radical attack. In recent years, however, it has become increasingly apparent that radiation can act via a variety of untargeted (transacting) mechanisms, as demonstrated by the very high frequency events or effects manifest at a distance from the radiation track. A few of the many examples in vitro and in vivo experimental systems are: radiation induced instability⁴⁾, initiation of cell transformation²¹⁾, germ-line minisatellite mutations²²⁾, instability in unirradiated bystander cells²³⁾ and bystander expression of damage recognition/repair proteins. Little is known about the mechanisms of these various effects and there is a paucity of systematic data from which to infer high-LET mechanisms or to predict effectiveness. It is clear that some effects are greater for high-LET radiations (e.g., instability), indicating that in these cases track structure remains important (either spatially or temporally). If other untargeted effects arise from generalized minor damage, then for these, high-LET radiations would be expected to be less effective. Further systematic experimental data, including the use of microbeam irradiations, should in future lead to more understanding of these phenomena.

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