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Initial events in the cellular effects of ionizing radiations: clustered damage in DNA

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Abstract. General correlations are found between the detailed spatial and temporal nature of the initial physical features of radiation insult and the likelihood of final biological consequences. These persist despite the chain of physical, chemical and biological processes that eliminate the vast majority of the early damage. Details of the initial conditions should provide guidance to critical features of the most relevant early biological damage and subsequent repair. Ionizing radiations produce many hundreds of different simple chemical products in DNA and also multitudes of possible clustered combinations. The simple products, including single-strand breaks, tend to correlate poorly with biological effectiveness. Even for initial double-strand breaks, as a broad class, there is apparently little or no increase in yield with increasing ionization density, in contrast with the large rise in relative biological effectiveness for cellular effects. Track structure analysis has revealed that clustered DNA damage of severity greater than simple double-strand breaks is likely to occur at biologically relevant frequencies with all ionizing radiations. Studies are in progress to describe in more detail the chemical nature of these clustered lesions and to consider the implications for cellular repair. It has been hypothesized that there is reduced repair of the more severe examples and that the spectrum of lesions that dominate the final cellular consequences is heavily skewed towards the more severe, clustered components.

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1. Introduction

The initial physical features of ionizing radiations are a major determinant of their final biological consequences. They set the starting conditions of atomic and molecular disturbance from which the subsequent chemical, biochemical and cellular modifications follow to either prevent, mitigate or establish permanent cellular damage. Despite this powerful chain of modifying factors that eliminate the vast majority of the initial disturbances, very clear and quite general correlations persist between the detailed spatial and temporal nature of the initial radiation insult and the likelihood of final biological consequences to the cell or even the whole body. Therefore, it is reasonable to look to this initial physical information to throw light on critical features of the early biochemical damage and subsequent cellular mechanisms in mammalian cells.

Amongst the generalities for many biological effects of interest is that cellular responses (such as inactivation, mutation, chromosome rearrangement, oncogenic transformation) tend to be stochastic, implying that they result from some rare minority of the bulk of radiation-induced molecular damage (in DNA or otherwise). Another general feature is that when the initial points of atomic disturbance occur closer together, as with more densely ionizing radiations, the likelihood of residual biological damage is enhanced, for a given absorbed dose (i.e. for approximately the same total number of ionizations). This is not a trivial observation because a consequence of closer ionizations is that fewer macromolecules are likely to be directly ionized and also fewer diffusing water radicals are likely to be available for reaction with macromolecules. An additional generality is that the probability of residual damage from densely ionizing radiations is much less open to modification by chemical, biochemical or other cellular factors than is that from the more sparsely ionizing radiations. Thus, there is a general indication that clustering of initial damage, in some form or other, is a biologically significant feature of ionizing radiations.

Such generalities may provide good guidance to both theoretical and experimental studies of the biologically most important pathways to residual damage.

2. Types of DNA damage

Ionizing radiations are known to produce a plethora of different types of molecular damage to DNA alone, and presumably also to other macromolecular species within irradiated cells. Table 1 gives estimates of the numbers of some of the early physical and biochemical damages that occur in a mammalian cells when irradiated with 1 Gy of low-LET radiation such as γ-rays. Yet, even for DNA, this is

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Table 1. Some of the damage in a mammalian cell nucleus from 1 Gy of low-LET radiation

Initial physical damage	
Ionizations in cell nucleus	~100 000
Ionizations directly in DNA	~2 000
Excitations directly in DNA	~2 000
Selected biochemical damage (Ward 1988)	
DNA single-strand breaks	1 000
8-Hydroxyadenine	700
T* (thymine damage)	250
DNA double-strand breaks	40
DNA–protein cross links	150
Selected cellular effects	
Lethal events	~0·2–0·8
Chromosome aberrations	~1
Hprt mutations	$\sim 10^{-5}$

by no means a complete or detailed list. Quoting from Hutchinson (1985): 'The major difficulty in studying the effects of ionizing radiation on DNA is the large number of products.... Thus, there are hundreds of kinds of radiation-induced products in DNA,' and this is just for simple products in DNA alone, without considering their possible clustered combinations or the influence of other molecules in the cellular environment!

It is unlikely that all of these various types of DNA damage will be equally important to the final cellular effects that are of interest for radiotherapy or for radiation protection. The numbers of the various damages are quite different, and repair processes are likely to operate with different rates and efficiencies, so some of the early damage types may be of trivial importance while others may dominate the radiobiological consequences. If many of the damage types are of comparable importance we are faced with a nearly impossible task of understanding their roles or to manipulate them to practical advantage other than on a crude phenomenological basis. By contrast, if only relatively few damage types dominate the biological consequences for human situations, there should be great rewards in identifying and focussing on these, rather than on those of little relevance even if much more abundant.

3. DNA strand breaks

Do DNA single-strand breaks (ssb) and simple base damages fall into this latter category of little relevance? The yield of ssb is about 10^3 cell^{-1} after 1 Gy of low-LET radiation, but this dose typically leads to <1 lethal event on average and a smaller or comparable number of chromosome aberrations. Induced *hprt* mutation frequencies are typically $\sim 10^{-5}$ Gy^{-1} (Thacker 1992), but an order of magnitude more ssb are produced directly in the 0·75 kb coding sequences of the gene itself, let alone its other associated functional sequences or its introns totalling about 45 kb. Thus, ssb *per se* do not stand out as a strong candidate for radiobiologically critical damage.

However, ssb are produced in great abundance and it is important to try to distinguish whether they really are unimportant, or whether a very small fraction do sneak through the repair systems, perhaps by chance or by virtue of their particular positions or associations with other damage, to cause significant phenotypic changes. Also, might an initial burden of ssb activate processes in a cell that persist and have consequences long after the ssb themselves have been repaired, for example via induced expression of early response genes? Optimum strategies to exploit or mitigate radiation effects could be very dependent on the answers to such questions.

Numerous additional arguments have been put forward against the importance of simple ssb. These include poor correlation with repair rates, radiosensitivity of different normal and mutant cells and dependence on radiation quality. It is now widely assumed in the literature and in research strategies that ssb are not of particular importance. Similar arguments have tended to be used also against other simple single-strand lesions such as isolated base damages.

Over the past two decades emphasis has shifted to DNA double-strand breaks (dsb) as *the* critical damage for radiobiological effects. Yet, dsb are not entirely immune to similar lines of argument as above. Clearly most of the initial dsb do not lead to observable cellular consequences (Table 1), correlation has not in general been found to be particularly good for numbers of initial dsb, or their repair, with radiosensitivity, and dsb show much less dependence on radiation quality than do most cellular effects of interest. So could it be that studies of dsb, too, as a general class of damage are misleading if we are aiming at the critical pathways of radiation effects?

4. Physical pointers to the biologically relevant types of damage

Ionizing radiation is unique amongst DNA-damaging agents in the broadness of the spectrum of damage that it can cause to DNA and other biomolecules. Therefore, it is worth looking back to the detailed nature of this initial physical insult and

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seeking guidance from the general experimental correlations between radiation quality and biological effectiveness to point towards those intermediate steps of biochemical damage and repair that are most relevant to cellular effects. We can now put aside some of the past simplistic and partisan views coming from particular disciplines. Chemists now know that the irradiated cell is not just a homogeneous sea of freely diffusing hydroxyl and other water radiolysis products originating from uniform 'spurs'. The high reactivity of the cellular environment allows only nanometre diffusion distances for reactive water radiolysis products (Roots and Okada 1975, Chapman and Gillespie 1981, O'Neill 1993) and therefore emphasizes the spatial structure of the radiation tracks with all their stochastic variability. Physicists now know that two ionizations, or specific amounts of energy, directly in the two DNA strands are neither necessary nor sufficient to produce a dsb or for final cellular effects. Scavengeable radicals from outside the DNA can make a substantial contribution, and all ionizations in DNA do not lead to a strand break. Biochemists know now that not all dsb are the same in type or in consequences and that classification of breaks into only ssb and dsb is too simplistic.

What pointers can the physics of radiation tracks give to the relevant pathways and damage types? Tracks can be described with great precision over cellular dimensions (micrometers) directly from experimental and theoretical information (e.g. Goodhead 1987). Over smaller dimensions, down to nanometers and comparable with DNA, track structure simulation methods can be applied (e.g. Goodhead 1987, Paretzke 1987). While these simulations are as yet by no means perfect for atomic resolution in the inhomogeneous and condensed environment of cellular DNA, they can provide a wealth of relevant information to allow systematic investigation of the variation of radiation quality for comparison with biological effectiveness.

The initial insult to cells from virtually all ionizing radiations used in radiobiology is in the form of highly structured tracks. Track structure analyses show that most small molecules or portions of a large molecule are very unlikely to experience any direct ionization at all or to be within diffusion distance in the cellular milieu of any primary radical at doses of practical relevance. For example, a 6-bp segment of DNA in a γ-ray irradiated cell has a probability of only about $10^{-6}\,Gy^{-1}$ of suffering a direct ionization and about $10^{-4}\,Gy^{-1}$ of being within 4 nm of any ionization or primary radical (Goodhead and Nikjoo 1989, Nikjoo *et al.* 1991). Thus, if more than one point of ionization or radical attack is to cause a dsb this will almost inevitably be from associated ionizations occurring stochastically within a *single* track rather than from two distinct tracks.

It is well known that for most cellular effects of interest in mammalian cells concentrating the ionizations increases the biological effectiveness of a given absorbed dose of radiation (at least up to conditions of quite extreme concentration) (e.g. Figure 1). This is despite the fact, confirmed quantitatively by track structure evaluations (Goodhead and Nikjoo 1989), that fewer DNA segments or other such molecules should then suffer any radiation damage at all. The enhanced effectiveness per unit absorbed dose of more densely ionizing radiations persists right through to *in vivo* effects such as cancer induction or tumour sterilization. Relatively biological effectiveness (RBE) values for high LET radiations compared with low-LET have been found to be typically from about 3–>50 depending on cell type, biological effect and irradiation conditions. What types of biochemical or intermediate damage in the cells follow this trend?

Consider first single-radical products, either in water or DNA, from a single ionization or excitation. In this case the yield clearly has totally the wrong dependence on LET (e.g. Figure 2), the yield *decreasing* as ionization density is increased. Single-strand breaks, too, have such a dependency (Charlton *et al.* 1989). By contrast, yields of molecular products from *pairs* of radicals in water can show a tendency to increase with increasing LET (e.g. Figure 2). This contrasting dependency is a natural consequence of bringing more primary radicals closer together in the radiation track—they are then more likely to react with one another to increase the molecular yield and also to decrease the yield of escaping single radicals.

Double-strand break induction in DNA in oxygenated mammalian cells seems to show little dependence on LET. Recent experimental measurements have shown RBEs of $\lesssim 1$ for neutrons (Prise *et al.* 1987, Peak *et al.* 1991), slow protons (Prise *et al.* 1990, Jenner *et al.* 1992), and even for slow α-particles (Coquerelle *et al.* 1987, Prise *et al.* 1987, Fox and McNally 1990, Jenner *et al.* 1993) of LETs that are in the region of maximum RBE for most cellular effects as in Figure 1. Some earlier studies did indicate larger RBEs of 1·6 (Blöcher 1988) or 3·5 (Kampf and Eichhorn 1983). Absence of strong dependence on LET suggests that dsb do not result predominantly from single ionizations (single radicals), for which reduction in RBE with increasing LET would be expected, nor from *large* clusters of closely adjacent ionizations, for which larger rises in RBE would be expected. The observation that RBEs

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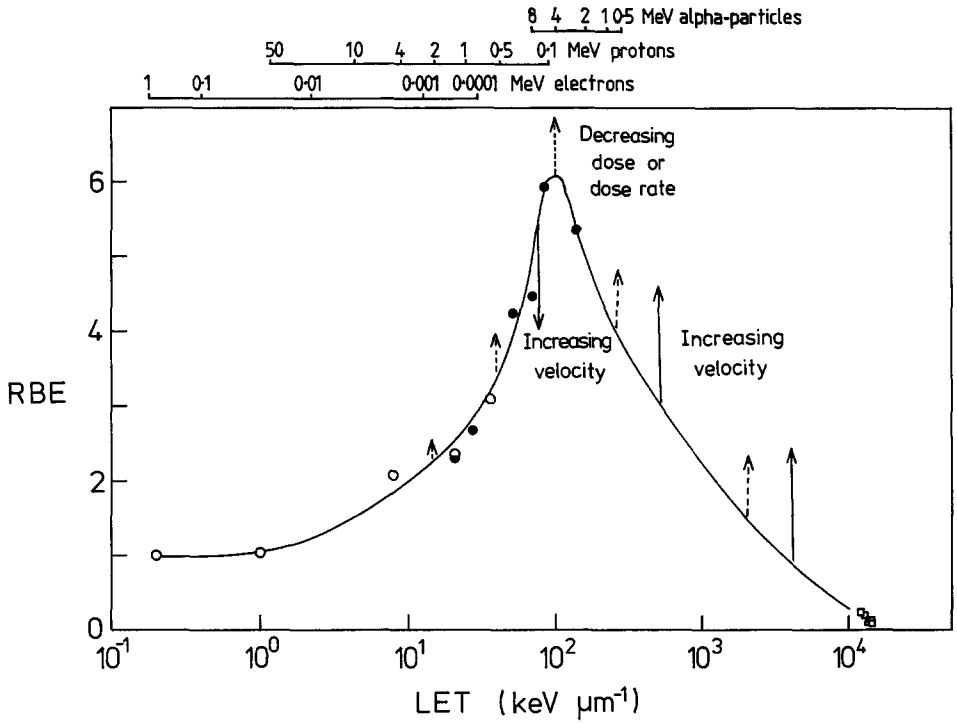

Figure 1. Example of dependence of RBE on unrestricted LET, in this case for inactivation of 50% of a population of irradiated hamster cells by photons and charged particles (adapted from Goodhead 1987). The indicated dependence on increasing velocity of the radiation particles (solid arrows) is due to greater dispersion of the ionizations by longer-ranged δ-rays; the dependence on dose and dose-rate (broken arrows) is due mainly to nonlinear dose dependence of the low LET reference radiation. Unrestricted LET covered by radiations of common interest are shown by bars above the diagram, for electrons (as produced by X- and γ-rays), protons (as recoils in neutron irradiations) and α-particles (as from radionuclide decays) of the indicated energies.

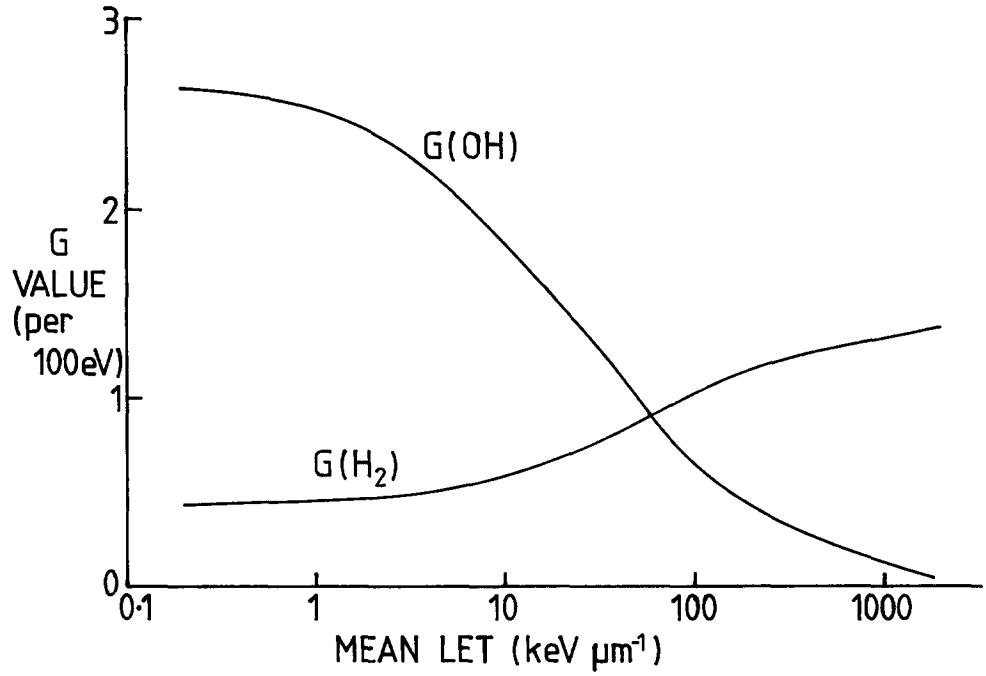

Figure 2. Yield of a single radical product (OH) compared with a biradical molecular product (H_2) in their dependence on LET in water radiolysis by γ-rays and charged particles (redrawn from Burns and Sims 1981).

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are about unity for these slow high-LET ions implies that most measured dsb result from quite modest local clusters within single tracks (Charlton *et al.* 1989, Goodhead and Nikjoo 1989, Brenner and Ward 1992, Michalik 1992). Some of these analyses may have tended to overestimate the required cluster sizes, on the basis of the earlier experimental data that indicated larger RBEs.

These small RBEs for dsb leave unexplained the molecular nature of the damage responsible for the much larger RBEs observed for cellular effects. For example, for α-particles of about $120\,\mathrm{keV}\,\mu\mathrm{m}^{-1}$, RBEs of $\lesssim 1{\cdot}0$ have been measured for dsb induction in V79-4 hamster cells (Jenner *et al.* 1993), but RBEs for cell inactivation and *hprt* mutation have been found to be up to >6 and >10 (depending on dose), respectively, in the same cell line (Thacker *et al.* 1982), consistent also with high RBEs for inactivation and mutation in other rodent and human fibroblastic cell types (e.g. Cox *et al.* 1977, Thacker 1992). It may be pertinent to note, too, that the measured RBE for breaks in prematurely condensed chromosomes in human fibroblasts with these α-particles was 2·2 (Bedford and Goodhead 1989), considerably smaller than the RBEs for inactivation ($\sim 4{\cdot}0$) or mutation ($\sim 7{\cdot}1$) of these cells (Cox *et al.* 1977) but larger than the above RBE for dsb. It seems that numbers of dsb, as a homogeneous class of DNA damage, are not the predominant determinant of cellular effectiveness.

The higher RBEs are likely to arise only from damage of somewhat greater complexity either (1) at the level of the individual small damaged segments of DNA, (2) in the higher-order DNA associations with protein, etc., (3) in the spatial association of separate dsb, or (4) in the overall simultaneous burden to the entire cell nucleus (in $\lesssim 10^{-12}$s). In all these respects there are substantial differences between high- and low-LET radiations by virtue of their track structures. Which one, or more, of these factors is responsible for radiation quality effects has significant implications for understanding mechanisms of biological action of radiation quite generally, including practical aspects even for low-LET radiations in therapy and at protection levels. The rest of this paper will concentrate on the first of the above, i.e. at the level of individual small segments of DNA and their immediate surroundings. Arguments beyond the scope of the present paper can be put forward to suggest that some of the other three factors may be less relevant.

5. Clustered damage in DNA

Consider briefly what the radiation track structure may be able to tell about likely types of molecular damage. Individual radiation tracks can be simulated very well in water vapour and, with somewhat greater uncertainties, in liquid water and other condensed materials (e.g. Paretzke 1987, Turner *et al.* 1982, Terrissol and Beaudre 1990, Uehara *et al.* 1993). These Monte Carlo simulations produce complete individual histories of tracks at the level of every individual atomic or molecular interaction (ionization, excitation, etc.) of the primary radiation particles and all their secondary electrons. The consequent spatial patterns (track structures) should be very reliable as accurate and true descriptions of real tracks in living cells when viewed at a resolution of tens of nanometres or greater. At higher resolution there is more uncertainty but even over a few nanometres the general patterns, main features and comparisons should have substantial validity. Detailed analysis at nanometre or subnanometre resolution requires more cautious interpretation. Important aspects of ongoing research are to reduce uncertainties, improve resolution and understand differences between existing simulation codes (Paretzke *et al.* 1986, Nikjoo *et al.* 1993). Existing Monte Carlo codes represent an enormous advance over the previously available descriptions such as those based on stopping power, average track profiles or simple random energy loss along the track.

The literature contains a number of displayed examples of actual simulated tracks, necessarily plotted for clarity as two-dimensional projections (e.g. Paretzke 1987, Goodhead and Nikjoo 1989, Nikjoo *et al.* 1989). These should be distinguished clearly from the many schematic representations that different authors have sketched to describe concepts and illustrate typical track features; these are seldom accurate. Basic problems in preparing any visual display of radiation tracks are the need for two-dimensional projection of three-dimensional tracks, the inability of any few tracks to represent the enormous stochastic variability between individual tracks from even identical incoming particles, and the impossible task of simultaneously displaying the large-scale features of tracks over cellular dimensions and the more microscopic features over say DNA dimensions. This latter problem is exacerbated by the fact that a major feature of all radiations is the abundance within the tracks of very low-energy secondary electrons whose entire substructures are confined to tens of nanometres or less, usually with substantial clustering of ionizations therein. Hence, distorted schematic illustrations such as in Figure 3

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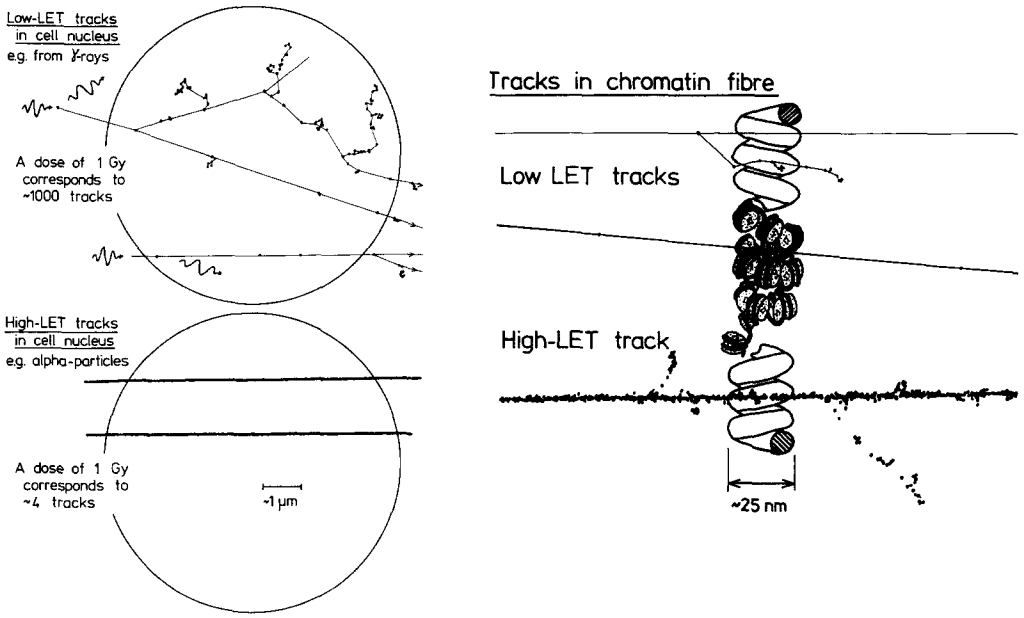

Figure 3. Schematic representation of (left) a cell nucleus irradiated with two electron tracks from γ-rays (low LET) or two α-particle tracks (high LET). A true scale diagram would require that the clusters of ionizations near the track ends of the low-energy secondary electrons be very much more compact, illustrated (right) on the scale of DNA and chromatin fibre (reproduced from Goodhead 1988).

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have had to be used, with appropriate caveats to warn the reader that the small and large features are not to scale.

For the present purpose we are interested to estimate from the accurately simulated track structures what types and frequencies of DNA damage might be produced by ionizing radiations. Consider a segment of DNA, such as on the left of Figure 4, and randomly superimpose on it and its surroundings a randomly selected simulated track from the given type of radiation. This must be repeated many thousands of times to obtain statistically meaningful results (Charlton *et al.* 1985, Nikjoo *et al.* 1989, 1991). It is found that the most likely occurrence in, or near, the DNA is a single ionization or a single excitation, for any radiation type although particularly so for low-LET radiations. But much more dramatic events do also occur even with low-LET radiations such as X- or γ-rays (Goodhead and Nikjoo 1989, Nikjoo *et al.* 1991). To illustrate the possibilities, in the upper centre of Figure 4 and drawn to scale is a portion of a simulated track showing a low-energy electron; such electrons are produced in abundance in almost all irradiations (Paretzke 1987, Nikjoo and Goodhead 1991). When this particular track is superimposed randomly onto the DNA, or very near to it, it is easy to envisage that the consequence might be quite complex combinations of strand breaks (from ionizations directly in the sugar–phosphate moiety and/or from nearby hydroxyl radical attack) and base damages (also from direct ionizations and/or radical attack). One such possibility is illustrated on the upper right of Figure 4. In current experimental assays of DNA breaks this would be counted simply as a 'dsb' and not distinguished in any way from a simple clean nick in each strand such as from a restriction enzyme. But surely the cells's recognition and repair systems are likely to see this clustered DNA damage as substantially different?

The reader is invited to photocopy this example electron track, randomly overlay it on the DNA and imagine the varieties of possible DNA damage. But bear in mind that every electron track has different structure, so the possible damages are even more diverse.

Also shown in Figure 4 is a very short portion of the track of a 4 MeV α-particle. In this particular portion the secondary δ-ray electrons all happen to travel only a very short distance from the α-particle. But on occasions individual δ-rays can receive sufficient energy to travel more than a hundred nanometres. Also, the α-particle can by chance travel several nanometres without interacting at all thus passing right through the DNA without directly affecting it.

Again the reader is invited to overlay this track randomly onto the DNA. Some of the possible

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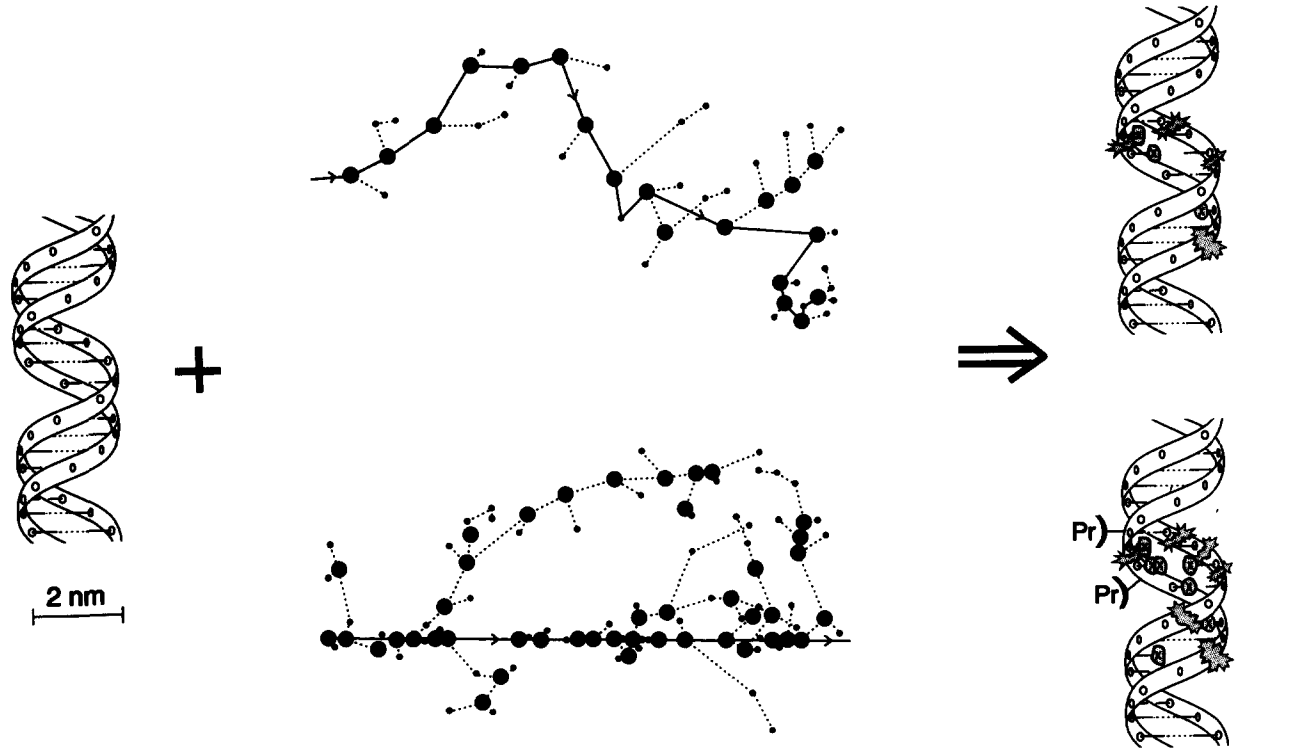

Figure 4. A segment of DNA drawn on the same scale as two-dimensional projections of an actual simulated low-energy electron track (upper: initial energy 500 eV and coming to rest) and an actual simulated short portion of an α-particle track (lower: energy 4 MeV). Superimposing these tracks randomly onto the DNA suggests that a variety of clustered damages can result; two notional possibilities are illustrated on the right. Broken ribbons indicate DNA strand breaks, X indicates base damage and Pr indicates a DNA–protein cross link. In these track diagrams large circles are ionizations and small circles are excitations.

configurations of DNA damage that now emerge may be quite startling. The one shown (lower right) includes also the possibility of crosslinks to adjacent damaged protein. DNA–DNA crosslinks are also possible. Such very severe, highly clustered damage as that illustrated must surely be seen by the cell as different from a simple clean dsb and be likely to have different consequences. However, many of the possible damage configurations from random positioning of the α-track will be no different from those that might arise from low-LET radiations. Further examples of ion and electron tracks can be seen in publications cited above, but no small number of such samples can be regarded as truly representative of the stochastic diversity of track structures.

Even from the above few qualitative illustrations with actual simulated tracks drawn to scale, it seems reasonable to conclude that initial clustered damage in DNA *can* result from all ionizing radiations and that these can be considerably more severe than simple dsb. It is reasonable also to suggest that such damage may be more difficult for the cell to repair and even that some of the more severe examples may be essentially unrepairable with full fidelity.

This leads then to the essential question as to whether such types of clustered damage do in actuality occur sufficiently often for them to have a realistic, or even dominant, role in the biological effects of radiation. Conversely, could it be that these spatial coincidences of clustered ionizations on or near to DNA, and the consequent clustered DNA damage, are so rare to be of no real relevance at any doses of practical interest or by comparison with the more abundant minor points of damage? Such questions could ideally be addressed by quantitative theoretical evaluation, including details of the competing chemical pathways (O'Neill 1993), of sufficiently large numbers of individual tracks randomly superimposed on sufficient DNA molecules in a simulated cellular environment. Many thousands of tracks and DNA segments would need to be analysed to obtain statistically robust results. This has not been achieved to date although serious efforts are now underway within the uncertainties and limits on resolution of available track structure codes and current knowledge of the intermediate chemical processes leading from initial ionizations and excitations to stable changes to the DNA.

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But there is already quite sufficient quantitative information to indicate that substantial clustering of initial ionizations and excitations does indeed occur in, and near to, DNA with sufficiently high frequencies to be of likely relevance to cellular effects. By considering simple volume targets of DNA, with or without inclusion of surrounding water or protein, superimposed on simulated radiation tracks, it has been possible to evaluate absolute frequency distributions of energy deposition by individual tracks in single target volumes placed randomly in the cell (assumed to be water) irradiated by any one of a large number of radiations. From these data direct estimates can be made of the numbers of times that clusters should occur in or near to DNA in cells receiving any given dose (e.g. Goodhead *et al.* 1985, Goodhead and Nikjoo 1989, Brenner and Ward 1992, Michalik 1992). The results (e.g. Figure 5) show that even for low-LET radiations quite severe clusters should occur at moderate frequencies directly in the DNA alone, and considerably more so if somewhat larger volumes are considered so as to include surrounding water or protein. With high-LET radiations there are higher frequencies of more severe clusters, including some that are uniquely severe (Figure 5). But even with slow α-particles the

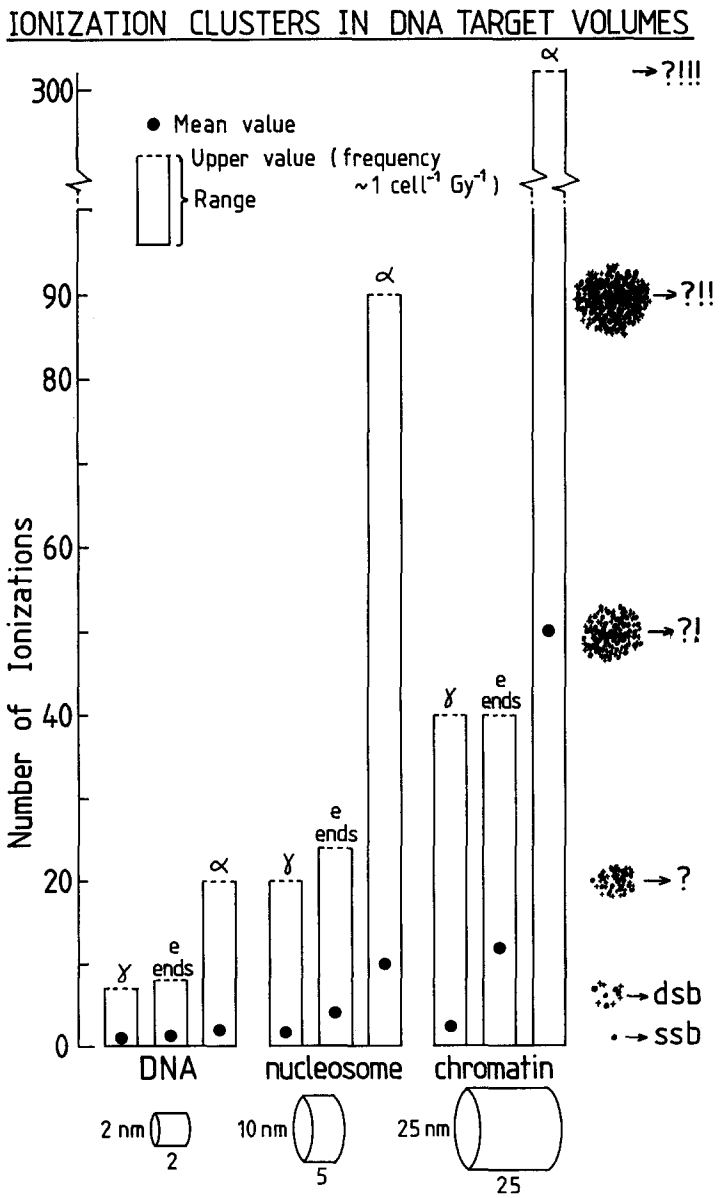

Figure 5. Sizes of ionization clusters produced in DNA-related volumes by γ-rays, 1 keV electron track ends and slow α-particles. There are on average similar, or slightly larger, numbers of excitations also in the clusters (from Goodhead 1992).

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full spectrum of low-LET-type clusters are included; for all radiations sparse ionization or small clusters are the most frequently occurring.

For the above estimates of initial clustered damage, track scoring has been based on *energy* deposition in the target volumes, rather than counting numbers of ionizations (and excitations). This was so that results and comparisons should be less susceptible to choice of vapour/liquid and material and to other uncertainties in applying the existing track structure simulation codes to DNA-related volumes in the cellular environment. Similar general features are, of course, found when ionization clusters are counted (Wilson and Metting 1988, Brenner and Ward 1992, Michalik 1992) since it is the ionizations and excitations that constitute energy deposition. In order to proceed to full chemical simulation of the consequences of the physical clusters the fate of individual ionizations (and excitations) in particular atoms within the molecules of DNA and its surroundings will have to be followed. However, from the existing information it already seems reasonable to expect that clustered damage in DNA is likely to occur at sufficiently high frequencies to present significant substrate for the cellular recognition and repair systems, including damage such as drawn in Figure 4.

6. Cellular response to clustered damage

It seems reasonable to hypothesize that cells will be best able to repair the more minor DNA damage and that perfect repair will become less probable for increasingly complex damage (Goodhead *et al.* 1980, 1985, Ward 1988, Goodhead 1989, 1992). Goodhead *et al.* (1993) have illustrated this in terms of severity of damage and suggested how reducing repairability can result in the residual cellular consequences being dominated by less frequent, but more severe, components of the initial spectrum of damage. Some clustered components may even be of such severity that they cannot ever be fully restored by the normal repair processes.

There are some lines of experimental evidence that support these theoretical evaluations and hypotheses. For example, there is the general tendency for cellular consequences of high-LET radiations to be less modifiable than those of low-LET radiations, by factors such as dose-rate, oxygenation, sensitizers, repair competence of the cell, etc. Such observations fit readily with the suggestion that the biologically dominant components of residual damage of high LET radiations have little chance, in any case, of being repaired; minor modifications of the initial damage or of the repair environment would not be expected to influence greatly the repair probabilities and cellular consequences of this already severe damage. Further evidence is provided by some notable observations in relation to DNA damaged, assayed as 'dsb' in conventional ways. First, there is apparently less cellular repair of α-particle-induced 'dsb' than of 'dsb' from γ-rays (Jenner *et al.* 1993); this suggests reduced repairability of complex 'dsb' from more severe ionization clusters. Second, the number of measured 'dsb' per lethal event has been found to vary in a single cell line from about 20 (for slow α-particles) to nearly 100 (for fast electrons and hard X-rays) and many hundreds (for hydrogen peroxide) depending on the agent inducing the 'dsb' (Fox and Prise 1992). This is strongly suggestive of 'dsb' of widely differing complexity and hence repairability.

7. Conclusions

There is now subsantial evidence suggesting the biological importance of clustered lesions at the DNA level in irradiated cells. Advancing theoretical and experimental methods should be able to test these hypotheses further and establish clearly their relevance to the biological effects of radiation. On the theoretical side detailed chemical pathways need to be incorporated into simulations of initial clustered ionizations within a track leading to clustered molecular damage in DNA. In doing this, uncertainties in the track structure codes themselves and their applicability in the cellular milieu need to be considered. On the experimental side new methods are needed to resolve the diversity of damage complexity that is grouped as 'dsb' by current assays, and also to identify other possible types of clustered damage including DNA associations with protein or other molecules that may add to the severity, and to follow the consequences of these damages through cellular repair to final effects.

References

BEDFORD, J. S. and GOODHEAD, D. T., 1989, Breakage of human interphase chromosomes by alpha-particles and X-rays. *International Journal of Radiation Bology*, **55,** 211–216.

BLÖCHER, D., 1988, DNA double-strand break repair determines the RBE of α-particles. *International Journal of Radiation Biology*, **54,** 761–771.

BRENNER, D. J. and WARD, J. F., 1992, Constraints on energy deposition and target size of multiply-damaged sites associated with DNA double-strand breaks. *International Journal of Radiation Biology*, **61,** 737–748.

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BURNS, W. G. and SIMS, H. E., 1981, Effect of radiation type in water radiolysis. *Journal of Chemical Society, Faraday Transactions*, **77,** 2803–2813.

CHAPMAN, J. D. and GILLESPIE, C. J., 1981, Radiation-induced events and their time-scale in mammalian cells. *Advances in Radiation Biology*, **9,** 143–198.

CHARLTON, D. E., GOODHEAD, D. T., WILSON, W. E. and PARETZKE, H. G., 1985, The deposition of energy in small cylindrical targets by high LET radiations. *Radiation Protection*, **13,** 123–125.

CHARLTON, D. E., NIKJOO, H. and HUMM, J. L., 1989, Calculation of initial yields of single and double-strand breaks in cell nuclei from electrons, protons and alpha-particles. *International Journal of Radiation Biology*, **56,** 1–19.

COQUERELLE, T. M., WEIBEZAHN, K. F. and LÜCKE-HUHLE, C., 1987, Rejoining of double-strand breaks in normal human and ataxia-telangiectasia fibroblasts after exosure to ^{60}Co γ-rays, ^{241}Am α-particles or bleomycin. *International Journal of Radiation Biology*, **51,** 209–218.

COX, R., THACKER, J., GOODHEAD, D. T. and MUNSON, R. J., 1977, Mutation and inactivation of mammalian cells by various ionizing radiations. *Nature (London)*, **267,** 425–427.

FOX, J. C. and MCNALLY, N. J., 1990, The rejoining of DNA double-strand breaks following irradiation with ^{238}Pu α-particles: evidence for a fast component of repair as measured by neutral filter elution. *International Journal of Radiation Biology*, **57,** 513–521.

FOX, J. C. and PRISE, K. M., 1992, DNA lesions: linear energy transfer and radiosensitive mutants. In: *Radiation Science: Of Molecules, Mice and Men*. Edited by: H. Denekamp and D. G. Hirst. *British Journal of Radiology*, Suppl. 24, pp. 48–52.

GOODHEAD, D. T., 1987, Relationship of microdosimetric techniques to applications in biological systems. In: *The Dosimetry of Ionising Radiations*, Vol. II. Edited by: K. R. Kase, B. E. Bjarngaard and F. H. Attix (Orlando, FL, Academic), pp. 1–89.

GOODHEAD, D. T., 1988, Spatial and temporal distribution of energy. *Health Physics*, **55,** 231–240.

GOODHEAD, D. T., 1989, The initial physical damage produced by ionizing radiations. *International Journal of Radiation Biology*, **56,** 623–634.

GOODHEAD, D. T., 1992, Radiation tracks in biological materials: initial damage in cells, DNA and associated structures. In: *Genes, Cancer and Radiation Protection* Edited by: M. L. Mendelsohn (Bethesda, MD, National Council on Radiation Protection and Measurements), pp. 25–37.

GOODHEAD, D. T., CHARLTON, D. E., WILSON, W. E. and PARETZKE, H. G., 1985, Current biophysical approaches to the understanding of biological effects of radiation in terms of local energy deposition. In: *Proceedings of the Fifth Symposium on Neutron Dosimetry*. Edited by: H. Schraube, M. Burger and J. Booz (Luxembourg, Commission of the European Communities), EUR 9762 En, pp. 57–68.

GOODHEAD, D. T., MUNSON, R. J., THACKER, J. and COX, R., 1980, Mutation and inactivation of cultured mammalian cells exposed to beams of accelerated heavy ions. IV. Biophysical interpretation. *International Journal of Radiation Biology*, **37,** 135–167.

GOODHEAD, D. T. and NIKJOO, H., 1989, Track structure analysis of ultrasoft X-rays compared to high- and low-LET radiations. *International Journal of Radiation Biology*, **55,** 513–529.

GOODHEAD, D. T., THACKER, J. and COX, R., 1993, Effects of radiations of different qualities on cells: molecular mechanisms of damage and repair. *International Journal of Radiation Biology*, **63,** 543–556.

HUTCHINSON, F., 1985, Chemical changes induced in DNA by ionizing radiation. *Progress in Nucleic Acid Research and Molecular Biology*, **32,** 115–154.

JENNER, T. J., BELLI, M., GOODHEAD, D. T., IANZINI, F., SIMONE, G. and TABOCCHINI, M. A., 1992, Direct comparison of biological effectiveness of protons and alpha-particles of the same LET. *International Journal of Radiation Biology*, **61,** 631–637.

JENNER, T. J., DE LARA, C. M., O'NEILL, P. and STEVENS, D. L., 1993, The induction and rejoining of DNA double strand breaks in V79-4 mammalian cells by γ- and α-irradiation. *International Journal of Radiation Biology*, **64,** 265–273.

KAMPF, G. and EICHHORN, K., 1983, DNA strand breakage by different radiation qualities and relation to cell killing: further results after the influence of α-particles and carbon ions. *Studia Biophysica*, **93,** 17–20.

MICHALIK, V., 1992, Model of DNA damage induced by radiations of diffeerent quality. *International Journal of Radiation Biology*, **62,** 9–20.

NIKJOO, H. and GOODHEAD, D. T., 1991, Track structure calculations illustrating the prominent role of low energy electrons in radiobiological effects of low-LET radiations. *Physics in Medicine and Biology*, **36,** 229–238.

NIKJOO, H., GOODHEAD, D. T., CHARLTON, D. E. and PARETZKE, H. G., 1989, Energy deposition in small cylindrical targets by ultrasoft X-rays. *Physics in Medicine and Biology*, **34,** 691–705.

NIKJOO, H., GOODHEAD, D. T., CHARLTON, D. E. and PARETZKE, H. G., 1991, Energy deposition in small cylindrical targets by monoenergetic electrons. *International Journal of Radiation Biology*, **60,** 739–756.

NIKJOO, H., TERRISSOL, M., HAMM, R. N., TURNER, J. E., UEHARA, S., PARETZKE, H. G. and GOODHEAD, D. T., 1993, Comparison of energy deposition in small cylindrical volumes by electrons generated by Monte Carlo track structure codes for gaseous and liquid water. *Radiation Protection Dosimetry* (in press).

O'NEILL, P., 1993, The role of hydration and radiation quality in the induction of DNA damage–chemical aspects. *Advances in Space Research* (in press).

PARETZKE, H. G., 1987, Radiation track structure theory. In: *Kinetics of Nonhomogeneous Processes*. Edited by: G. R. Freeman (New York, Wiley-Interscience), pp. 89–170.

PARETZKE, H. G., TURNER, J. E., HAMM, R. N., WRIGHT, H. A. and RITCHIE, R. H., 1986, Calculated yields and fluctuations for electron degradation in liquid water and water vapour. *Journal of Chemical Physics*, **84,** 3182–3188.

PEAK, M. J., WANG, L., HILL, C. K. and PEAK, J. G., 1991, Comparison of repair of DNA double-strand breaks caused by neutron or gamma radiation in cultured human cells. *International Journal of Radiation Biology*, **60,** 891–898.

PRISE, K. M., DAVIES, S. and MICHAEL, B. D., 1987, The relationship between radiation-induced DNA double-strand breaks and cell kill in hamster V79 fibroblasts irradiated with 250 kVp X-rays, 2·3 MeV neutrons or ^{238}Pu α-particles. *International Journal of Radiation Biology*, **52,** 893–902.

PRISE, K. M., FOLKARD, M., VOJNOVIC, B., DAVIES, S., ROPER, M. J. and MICHAEL, B. D., 1990, The irradiation of V79 mammalian cells by protons with energies below 2 MeV. Part II: Measurement of oxygen enhancement ratios

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and DNA damage. *International Journal of Radiation Biology*, **58,** 261–277.

ROOTS, R. and OKADA, S., 1975, Estimation of life times and diffusion distances of radicals involved in X-ray-induced DNA strand breaks or killing of mammalian cells. *Radiation Research*, **64,** 306–320.

TERRISSOL, M. and BEAUDRE, A., 1990, Simulation of space and time evolution of radiolytic species induced by electrons in water. *Radiation Protection Dosimetry*, **31,** 171–175.

THACKER, J., 1992, Radiation-induced mutation in mammalian cells at low doses and low dose rates. In: *Advances in Radiation Biology*, Vol. 16. Edited by: O. F. Nygaard, W. K. Sinclair and J. T. Lett (San Diego, CA, Academic), pp. 77–124.

THACKER, J., STRETCH, A. and GOODHEAD, D. T., 1982, The mutagenecity of α-particles from plutonium-238. *Radiation Research*, **92,** 343–352.

TURNER, J. E., PARETZKE, H. G., HAMM, R. N., WRIGHT, H. A. and RITCHIE, R. H., 1982, Comparative study of electron deposition and yields in water in the liquid and vapour phases. *Radiation Research*, **92,** 47–60.

UEHARA, S., NIKJOO, H. and GOODHEAD, D. T., 1993, Cross-sections for water vapour for Monte Carlo electron track structure code from 10 eV to 10 MeV region. *Physics in Medicine and Biology*, **38,** 1841–1854.

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.

WILSON, W. E., and METTING, N. F., 1988, Microdosimetric aspects of 0·3- to 20-MeV proton tracks. *Radiation Research*, **115,** 389–402.

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