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Review

The link between low-LET dose-response relations and the underlying kinetics of damage production/repair/misrepair

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Abstract.

Purpose: To review current opinion on the production and temporal evolution of low-LET radiobiological damage.

Methods: Standard cell survival models which model repair/misrepair kinetics in order to quantify dose-response relations and dose-protraction effects are reviewed and interrelated. Extensions of the models to endpoints other than cell survival, to multiple or compound damage processing pathways, and to stochastic intercellular damage fluctuations are surveyed. Various molecular mechanisms are considered, including double strand breaks restitution and binary misrepair.

Conclusions: (1) Linking dose-response curves to the underlying damage production/processing kinetics allows mechanistic biological interpretations of observed curve parameters. (2) Various damage processing pathways, with different kinetics, occur. (3) Almost every current kinetic model, whether based on binary misrepair or saturable repair, leads at low or intermediate doses to the LQ (linear-quadratic) formalism, including the standard (generalized Lea-Catcheside) dependence on dose protraction. (4) Two-track (β) lethal damage is largely due to dicentric chromosome aberrations, but one-track (α) lethal damage is largely caused by other mechanisms, such as point mutations in a vital gene, small deletions, residual chromosome breaks, induced apoptosis, etc. (5) A major payoff for 50 years of radiobiological modelling is identifying molecular mechanisms which underly the broadly applicable LQ formalism.

1. Introduction

When ionizing radiation strikes a cell, DSB (DNA double strand breaks) and other lesions are produced within less than a millisecond. Thereafter some of the damage is processed more slowly, in enzymatic repair or misrepair reactions, whose outcome often determines the fate of the cell. This review is concerned with damage production, with damage kinetics (i.e. time-evolution), and with the implications of the kinetics for biological endpoints such as clonogenic cell survival or chromosome aberrations. As was originally shown by Lea and others (e.g. Lea 1946, Haynes 1964, Kappos and Pohlit 1972), kinetic models of radiation damage production and processing can help unify and quantify radiobiological observations. The kinetic models give quantitative predictions for dose-response relations; and they lead to unified explanations for phenomena which superficially seem unrelated, for example by using repair/misrepair kinetics to relate shoulders on acute survival curves with increased survival when a given dose is protracted by fractionation and/or low dose-rate delivery. Since Lea's time, such unified, kinetically-based quantifications have been central for radiobiology and for its main applications, to radiotherapy, carcinogenesis risk estimates, and biological dosimetry.

Linking dose-response relations to underlying damage production and processing mechanisms has been carried out mainly with radiobiological 'reaction-rate' models (Lea 1946), i.e. models which track the per-cell average number of DSB and other lesions in time by using the equations of ordinary chemical kinetics for production, repair, and misrepair rates. Examples are the RMR (repair-misrepair) model (Tobias *et al.* 1980) and the LPL (lethal-potentially-lethal) model (Curtis 1986), both of which emphasize binary misrepairs, such as the production of a lethal dicentric chromosome aberration by the interaction of two DSB (Figure 1). Other reaction-rate models (e.g. Kiefer 1988b) consider saturable repair, corresponding to enzyme systems that can be overloaded. This review will show that radiobiological reaction-rate models give mechanistic biological interpretations to measured dose-response relations and dose-response parameters.

It appears that almost all radiobiological reaction-rate models lead approximately to linear-quadratic (LQ) dose-response relations if the dose is not too high or the dose delivery is sufficiently protracted. The LQ approximation to these radiobiological reaction-rate

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¶Abbreviations: DSB = double strand break; LQ = linear quadratic; LPL = lethal-potentially-lethal; RMR = repair misrepair; PCC = premature chromosome condensation.

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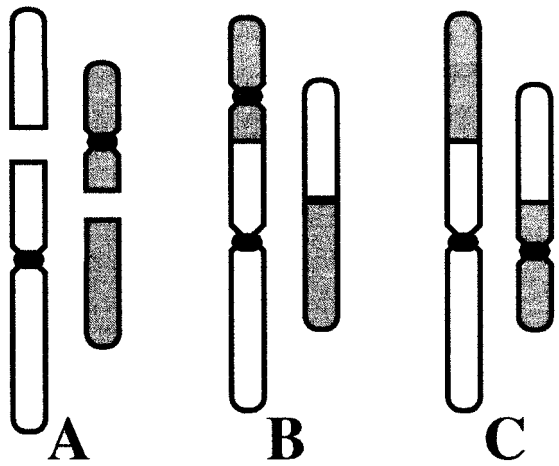

Figure 1. Examples of binary misrepairs. Figure 1A shows two chromosomes; each has one DSB, shown as a gap. Centromeres, which are needed for proper transmission of chromosomes to daughter cells at mitosis, are shown as black constrictions. Most DSB are restituted, but a few undergo binary misrepair. As shown in Figure 1B, a binary misrepair can make a dicentric (Cornforth and Bedford 1993). Typically this destroys the clonogenic viability of the cell. The dicentric is accompanied by an acentric fragment, and the two together are here counted as one lethal lesion. About half the time, the two DSB shown in Figure 1A lead to a translocation, shown in 1C. Translocations involve large scale rearrangements, and can sometimes cause dangerous alterations in cellular phenotype, but most do not impair cellular survival. They are non-lethal binary misrepairs. Both dicentrics 1B and translocations 1C are examples of exchange-type chromosome aberrations.

models is not merely a power series expansion in dose; it includes a standard (generalized Lea-Catcheside) factor for cell sparing by dose-protraction, whose form is the same among the different radiobiological reaction-rate models for any kind of fractionation and/or low dose-rate irradiation. The relation between the kinetic reaction-rate models and the LQ formalism is well known for the LPL model (Thames 1985, Curtis 1986, Thames and Hendry 1987). The relation also holds, as shown in Appendix A.6., for other binary misrepair reaction-rate models, and, surprisingly, even for typical saturable repair reaction-rate models (Appendix A.7.). Apart from its basis in mechanistic models, the LQ formalism, with its standard dependence on the time-pattern of dose delivery, has come into very wide use in the 1990s for practical reasons. It is frequently applied to survival or other endpoints *in vitro*, is especially important for iso-effect estimates in radiotherapy, and is often invoked in biodosimetry or risk estimation. The LQ formalism thus serves as a common meeting ground for many theories, experiments and applications.

This review will start by discussing production, restitution and binary misrepair of DSB (Section 2). Section 3 explains in detail one fairly representative radiobiological reaction-rate model. The model is temporarily singled out as an illustrative example, pending subsequent discussion of other reaction-rate models. Section 4 analyses survival curves for acute or protracted irradiation, using this representative model as an example. Section 5 discusses applications of radiobiological reaction-rate models to dicentric chromosome aberrations and discusses the relation of aberrations to survival. Section 6 briefly describes some generalizations: to multiple damage pathways; to additional endpoints; to spatially inhomogeneous reactions; and to stochastic process models which can track the temporal evolution of cell-to-cell fluctuations in damage. Appendices discuss other radiobiological reaction-rate models, applicable to damage pathways neglected in the representative model of Section 3. Sections 2–5 (in contrast to Section 6 and the Appendices) emphasize the reasoning behind models, rather than a catalogue of different models. The goal throughout is to illustrate robust general properties by selected special cases.

The reader who wants to follow all the derivations step by step will need some knowledge of ordinary differential equations and of the Poisson distribution. However, specifically mathematical arguments and results have been relegated to the Appendices, and the main points will be stated in intuitive and biological terms, so the mathematics can be skimmed over without essential loss of continuity.

Some related topics are omitted. Damage kinetics on short time scales, less than a minute or so, is not analysed. As far as long time scales are concerned, cell-cycle kinetics can strongly influence, and be influenced by, the kinetics of damage processing (e.g. Brenner *et al.* 1995, Hahnfeldt and Hlatky 1996, Zaider *et al.* 1996), but lack of space precludes any systematic review of this many-sided subject here, and the main weakness of the models reviewed is that they do not explicitly consider cell-cycle kinetic effects. Moreover, it will usually be assumed that a single radiation track has a negligible probability of making more than one DSB, and some of the discussion will not apply to high LET radiation or to soft X-rays.

The basic viewpoint of the review is that various damage production and processing pathways occur in an irradiated cell, so various kinetic reaction-rate models are required, the key question being which pathways are dominant for the biologically important endpoints, and the key simplification being that most pathways lead to approximately the same dependence of response on dose and on dose-protraction, given by the standard LQ formalism.

2. Double strand breaks

2.1. *DSB production*

Some of the most important repair and misrepair reactions involve DSB. Double strand breaks production by ionizing radiation is proportional to dose (Frankenberg-Schwager 1989, Ward 1990, Iliakis 1991). Probably there are qualitatively different kinds of DSB (Hagen 1989, Iliakis 1991, Steel 1991, Ward 1994, Michalik and Frankenberg 1996, Pfeiffer *et al.* 1996). Probably, as will be discussed, only a fraction of the ~40 DSB/Gy produced participate in reactions which can lead to lethality. The fraction could be randomly selected (Lea 1946) or be biologically defined, e.g. DSB made on linker DNA (Chatterjee and Holley 1991), and/or DSB which are expressed as breaks in premature chromosome condensation experiments (Cornforth and Bedford 1993), and/or DSB which are particularly 'severe' (Sachs and Brenner 1993), and/or DSB made on geometrically special stretches of DNA (Cornforth and Bedford 1993), and/or 'reactive' DSB with free ends that have moved apart (Chen *et al.* 1996, Radivoyevitch *et al.* 1997), etc.

2.2. *DSB processing*

After being produced, most DSB undergo restitution, where the two free ends of a DSB are rejoined to restore the overall continuity of a chromosome, though not necessarily the exact DNA base pair sequence (surveys in Hagen 1989, Hutchinson 1995). A small proportion of restitutions are (clonogenically) lethal (Figure 2B). Instead of being restituted some DSB undergo illegitimate reunion (Figure 1), a 'binary', 'pairwise' 'quadratic', 'dual', 'second order', 'cooperative' misrepair reaction. Binary misrepair of DSB can be clonogenically lethal, as when a dicentric chromosome aberration is formed (Figures 1B and 2C), but need not be (Figures 1C and 2D).

2.3. *Restitution kinetics*

DSB restitution is sometimes *first order* (Frankenberg-Schwager 1989), leading to mono-exponential DSB decay, i.e.

$$(\mathrm{A})\ dU/dt = -\lambda U \Rightarrow (\mathrm{B})\ U = U(0)\exp[-\lambda t] \quad (1)$$

where $U(t)$ denotes the average number of DSB per cell at time t after irradiation and λ is the first order restitution rate constant (i.e. $\lambda = \ln 2/t_{1/2}$, where $t_{1/2}$ is the DSB half-life, so that $1/\lambda$ is the mean lifetime of a DSB). The interpretation of equation (1A) is that during a short time dt the average number of restituted DSB is $\lambda U(t)dt$, proportional to the average number present and to dt. Often the observed decay of DSB number is not well approximated by the mono-exponential form equation (1B), being instead bi-exponential (reviews in Frankenberg-Schwager 1989, Iliakis 1991, Bryant 1995) with a fast component, and with a slower component corresponding approximately to $\lambda = 1\ \mathrm{h}^{-1}$, or even being multi-exponential (Foray *et al.* 1996). Such more complex behaviours suggest the presence of two or more different types of DSB and multiple or compound damage pathways, as analysed mathematically in Subsection 6.1 below.

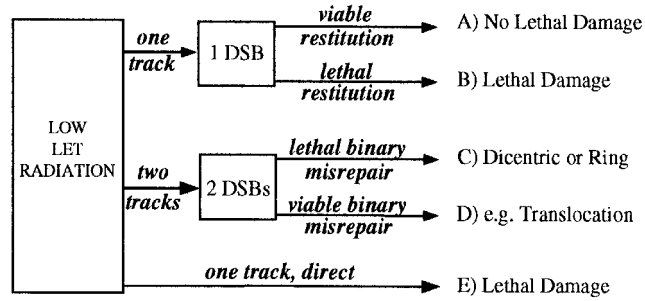

Figure 2. Some damage processing pathways. In the figure, 'track' refers to the subpicosecond deposition of energy caused by the passage of a charged or uncharged primary high energy particle (e.g. a gamma-ray photon) as well as all resulting secondary particles. Two different tracks are statistically independent, i.e. a 'track' is an 'event', in the terminology of microdosimetry. One-track or two-track action lead to different kinds of dose-response (Subsection 4.4). Most DSB are viably restituted (Figure 2A). In Figure 2B, the (clonogenically) lethal damage could be, for example, a small deletion within a vital gene. The molecular structure of a dicentric 2C was shown in Figure 1; some chromosome aberrations are rings, which for present purposes can be considered as two-track lethal binary misrepairs on exactly the same footing as dicentrics (Savage 1995). Binary misrepair can also be viable as shown in Figure 2D. Radiation damage is complex and, as discussed in Appendix A.1, there are different kinds of one-track lethal damage, shown generically in Figure 2E, in addition to lethal DSB restitution.

Equation (2A) is the simplest of all radiobiological reaction-rate models; the other kinetic models to be discussed all involve modifications of equation (1) to take into account, for example, binary misrepair contributing to the decay rate for DSB number (Subsection 3.1), and/or the temporal evolution of U if irradiation is protracted (Subsections 3.1 and 4.1), or saturated repair situations where the effective value of λ, instead of being constant, decreases with increasing U (Appendix A.7.), etc.

3. Repair and misrepair rates: a representative model

Throughout Sections 3 and 4 the endpoint is (clonogenic) cell survival but other endpoints are

considered in later sections. Many radiobiological models analyse the rate of cell killing for acute or protracted irradiation by using the differential equation methods of chemical kinetics. In Sections 3–5 one representative example is explained in detail, with representative applications. This model, whose equations are a special case of the RMR equations [Tobias *et al.* 1980], analyse first-order DSB restitution competing kinetically with binary DSB misrepair (Figure 2). These particular molecular mechanisms and the corresponding reaction-rate equations are here singled out for illustrative purposes, in order to analyse in depth specific examples of several features, such as kinetic competition between some kind of misrepair and some kind of repair, common to many different damage processing pathways. Other reaction-rate models, as well as interrelations among models, are surveyed later (Section 6; Appendices A.1. and A.3.–A.7.).

3.1. *Reaction-rate equations*

Assume a uniform population of many non-cycling cells which is irradiated with total dose D, delivered acutely or in a protracted regimen. Denote the dose rate at time t by $\dot{D}(t)$. Some simple examples of $\dot{D}$ are given in Appendix A.2. By choosing $\dot{D}$ appropriately one can describe any regimen, consisting of any number of acute doses, separated by any pattern of time intervals, and/or any continuous irradiation, at constant or variable dose-rate. It is assumed that cell sparing via different kinds of protraction, e.g. via low dose-rate irradiation or split dose irradiation, is due to essentially the same repair/misrepair phenomena, so that, barring cell-cycle kinetic complications beyond the scope of the present review, the basic formalism and parameters are the same, no matter what the form of the dose-rate function $\dot{D}$.

The representative model uses the per-cell average rates of DSB production, restitution, and binary misrepair to estimate the per-cell average rate of lethal lesion production and the surviving fraction of cells. The model starts by tracking DSB in time, using a generalization of the exponential decay equations (equation (1)). Denote the average number of DSB formed per unit dose by δ; for example one would expect $\delta = 40$ DSB per Gy (as a rough estimate) if all kinds of DSB are relevant to killing and expect δ to be smaller if only a subset of DSB is relevant (Section 2). The time rate of change of average DSB number $U(t)$ is taken as the sum of a DSB production term, a DSB repair term, and a DSB misrepair term, as follows (Tobias *et al.* 1980):

$$dU/dt = \delta\dot{D} - \lambda U - \kappa U^2 \qquad (2)$$

Here the three terms on the right are interpreted as follows. First, the dose delivered in the short time interval dt is $\dot{D}(t)dt$, so, in view of the definition of δ, the average per-cell number of DSB produced by irradiation during dt is $\delta\dot{D}dt$. Secondly, $\lambda U(t)$ is a DSB restitution rate, explained in detail under equation (1). Finally, the term $\kappa U^2(t)$ is a binary DSB removal rate, i.e. the average rate at which binary misrepairs remove DSB by using them in lethal lesions or in harmless rearrangements, with κ a rate constant (Lea 1946). For $U(t) \gg 1$, this U^2 dependence simply amounts to a reaction rate proportional to the square of the concentration, as in ordinary mass-action chemical kinetics for binary reactions (Erdi and Toth 1989). However, as shown in Appendix B.5., the appropriate rate is still κU^2 even if U is small, provided the statistical distribution of DSB from cell to cell is Poisson. At low LET this Poisson assumption is appropriate (Appendices B.5.–B.7.), though at high LET it can fail (Kellerer 1985, Goodhead 1987, Harder 1988, Albright 1989, Sachs *et al.* 1992).

Having specified the rate at which DSB are produced and disappear, the representative model next considers the rate at which cells acquire lethal lesions. Denote by $L(t)$ the average number of lethal lesions per cell. Then the per-cell average rate of forming lethal lesions is taken to be a sum of two terms, corresponding respectively to lethal restitution and lethal binary misrepair, as follows:

$$dL/dt = (1-\phi)\lambda U + (1/4)\kappa U^2 \qquad (3)$$

Here ϕ is defined as the proportion of restitutions which are viable (Tobias 1985; compare Figure 2A), so that, of the $\lambda U dt$ restitutions occurring during dt according to equation (2), $(1-\phi)\lambda U dt$ are lethal (Figure 2B); typically $1 - \phi \ll 1$. Assuming (as will be argued in Section 5) that lethal binary misrepair is predominantly dicentric formation, the factor of 1/4 in equation (3) can be motivated as follows. It takes two DSB, not just one, to make one dicentric (understood to be accompanied by an acentric fragment, Figure 1B). Moreover, on average binary misrepairs make about as many non-lethal translocations (Figure 1C) as they make lethal dicentrics (Sachs *et al.* 1997). Together these two factors correspond to the factor 1/4 for the κU^2 term in equation (3) compared to equation (2). This argument also works for rings as lethal binary misrepair products. The argument motivating the factor 1/4 ignores aberrations which are complex, an approximation which is appropriate at low and intermediate doses of low LET radiation, though not at high doses (Dutrillaux *et al.* 1985, Simpson and Savage 1996). At high doses, when complex aberrations are significant, the only way to handle the aberration kinetics, or to keep track of

what fraction is lethal (Savage 1995), is to use more detailed models, such as Monte-Carlo computer simulations (Subsection 6.5; Chen *et al.* 1996, 1997). Thus the factor of (1/4) is the best one can do within the framework of a simple reaction-rate model, but must be regarded with caution at high doses.

Conspicuously absent in equation (3) is a term proportional to $\dot{D}$, which would (Curtis 1986) reflect lethal lesions produced directly (Figure 2E). Such extra damage pathways are neglected in the present illustrative example but are extensively discussed later.

Equations (2) and (3) are differential equations which must be supplemented by initial conditions (Boyce and Diprima 1997). It is assumed that background DSB or lethal lesions are negligible and then appropriate initial conditions are either:

$$(\mathrm{A})\ U(0) = \delta D,\ L(0) = 0; \qquad \text{or } (\mathrm{B})\ U(0) = 0 = L(0) \tag{4}$$

(A) is appropriate for an acute dose D applied just before $t = 0$ (Appendix A.4.) and (B) is appropriate if all irradiation takes place after $t = 0$.

Equations (2)–(4) completely determine the per-cell average number $L(t)$ of lethal lesions at any time $t \geqslant 0$ (Boyce and Diprima 1997). However, $L(t)$ is not directly measured in a cell survival experiment. What is measured instead is the fraction, which will here be denoted by $S(t)$, of cells which have no lethal lesions whatsoever at time t. $S(t)$ can be approximated if one makes the assumption, appropriate at low LET, that the lethal lesions created during a short time dt, whose average number is $(dL/dt)dt$, are randomly distributed among cells, without regard for which cells already have lethal lesions. In that case, each hitherto surviving cell has (whether it likes it or not) a fair chance at getting one of the newly formed lethal lesions, i.e. $dS/dt = -(dL/dt)S$. Since $S = 1$ before radiation starts, the solution of this differential equation is (Boyce and Diprima 1997)

$$S(t) = \exp[-L(t)] \tag{5}$$

An alternative derivation of equation (5) is to use Poisson statistics for the lethal lesions (Appendices B.1. and B.5.). At high LET or for very high doses at low LET the relation between average per-cell lethal lesion number L and the fraction S of cells free from lethal lesions is more complicated than equation (5) (Appendices B.6. and B.7.).

3.2. *Fully developed endpoints*

In equations like equation (5) it is often convenient to take the limit $t \to \infty$ (e.g. Tobias 1985). This limit corresponds to a fully developed endpoint, i.e. repair and misrepair have run their full course. For example suppose that cells are irradiated during G_0, and that the G_1 phase of the cell cycle is long compared to the characteristic repair time $1/\lambda$. Then cell survival is a prototype of a fully developed endpoint (as is assaying chromosome aberrations at the next metaphase) and one can write the surviving fraction S as

$$S = S(\infty) \tag{6}$$

On the other hand, if, for example, damage fixation (Appendix A.5.) occurs within a short time after irradiation, then the endpoint is not fully developed.

3.3. *A representative model*

Equations (2)–(5) constitute a useful model, which illustrates the main features of radiobiological reaction-rate models in general. Explicit analytic solutions of these equations were given by Tobias *et al.* (1980) for the special cases of an acute dose or constant dose rate (Appendix A.3.); the equations can be solved numerically given any dose-rate function $\dot{D}$. There are many other radiobiological reaction-rate models (Section 6; Appendices A.1., A.3., A.4., and A.7.), but the model 3.1.1–3.1.4 is rather typical in its use of per-cell averages, its use of more than one lesion type, its assumption of lesion production linear in dose for arbitrary dose rates, its use of several reaction rates, etc. It will thus serve as a representative example. Its applications will now be discussed, emphasizing those features which it shares with other radiobiological reaction-rate models.

4. Survival curves

As shall be discussed, most radiobiological reaction-rate models predict virtually identical dose-response relations and dose-protraction effects at low and intermediate doses, given approximately by linear-quadratic (LQ) equations. At higher doses, different reaction-rate models make different predictions. Some details are now given on survival curves, using the representative model of Section 3 as an example.

4.1. *The LQ formalism*

The LQ formalism (Dale 1985, Thames 1985) is the simplest way to analyse acute and protracted dose delivery regimens systematically. It expresses surviving fraction S in terms of a damage coefficient α for lethal lesions made by one-track action (Figures 2B and/or 2E), a damage coefficient β for lethal lesions made by two-track action (e.g. Figure 2C),

and a repair rate λ similar to the DSB restitution rate in equations (1) or (2), as follows.

$$\ln S = -\alpha D - \beta G D^2 \tag{7}$$

where G, the generalized Lea-Catcheside dose-protraction factor, is given by

$$G = (2/D^2)\int_{-\infty}^{\infty} \dot{D}(t)\,dt \int_{-\infty}^{t} e^{-\lambda(t-t')}\dot{D}(t')\,dt' \tag{8}$$

This expression for G can be derived in various ways (Lea 1946, Kellerer and Rossi 1972, Chadwick and Leenhouts 1981, Dale 1985, Curtis 1986, Bedford and Cornforth 1987, Thames and Hendry 1987, Harder 1988, Nelson *et al.* 1989, Brenner *et al.* 1991). Appendix A.6. shows how equation (8) follows from the representative kinetic reaction-rate model of Section 3. G systematically accounts for the effects of protracting dose delivery in any way. A special case, which illustrates the general expression, equation (8), is for split-dose irradiation consisting of two acute doses D_1 and D_2 separated by time interval T. Then (Lea 1946)

$$G = \frac{D_1^2 + D_2^2 + 2D_1D_2e^{-\lambda T}}{D^2} \qquad \text{where } D = D_1 + D_2 \tag{9}$$

This special case, and two other simple special cases, are shown graphically in Figure 3. In general, as in these examples, $G \leqslant 1$, with $G = 1$ for a single acute dose. A small value of G corresponds to a large surviving fraction by equation (7) and the interpretation of $G < 1$ is cell sparing due to repair which occurs during continuous low dose-rate irradiation and/or between acute fractions. For example, the term $2D_1D_2\exp(-\lambda T)$ in equation (9) decreases as the time T between the two fractions increases, due to extra repair between fractions, quantified by the factor $\exp(-\lambda T)$.

During the last 15 years, the LQ formalism, defined by equations (7) and (8) has been applied to an extraordinarily broad spectrum of *in vitro* experiments on cell survival, using acute doses, split doses, or continuous low dose-rates; and it is currently very much the formalism of choice for calculating iso-effect doses in radiotherapy (Fowler 1989, Withers 1992). One of its main advantages is that there are only three adjustable parameters, in contrast to other models, whose use of four (or even more) adjustable parameters can easily lead to serious confusion.

4.2. *The LQ formalism as an approximation*

The model of Section 3 reduces to the LQ formalism providing that two restrictions hold: first,

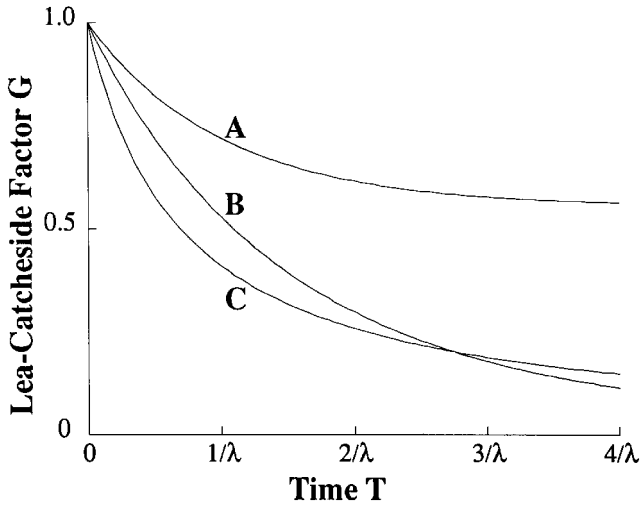

Figure 3. Examples of the generalized Lea-Catcheside dose-protraction factor G. G specifies how repair during any regimen of protracted irradiation decreases the effects of lethal misrepair. (A) is for an acute dose D_1 followed after time T by acute dose $2D_1$ (Equation (9) with $D_2 = 2D_1$). (B) Is for long-term irradiation by a decaying radioactive source with half-life T. The curve was obtained by inserting equation (A.2B) into equation (8) and integrating. (C) is for constant dose-rate irradiation, i.e. $\dot{D} = D/T$ for $0 \leqslant t \leqslant T$, where integrating equation (8) gives (Lea 1946)

$$G = [2/(\lambda T)^2)][e^{-\lambda T} - 1 + \lambda T]$$

Each kind of dose-protraction has its own pattern of cell sparing, but in all cases $G \leqslant 1$, with $G = 1$ for a single acute dose.

a large majority of DSB are removed by restitution rather than by binary misrepair; and secondly, survival is determined after misrepair has run its full course. More specifically, take surviving fraction S to be the fraction of cells without lethal lesions at large times, so that $S = \exp[-L(\infty)]$; now suppose that in equation (2) DSB restitution dominates DSB misrepair at all times, i.e. $\lambda U(t) \gg \kappa U^2(t)$; and finally define the LQ parameters α, β, λ using the parameters $\delta, \phi, \kappa, \lambda$ of the representative model in Section 3 by

$$\alpha = \delta(1-\phi), \qquad \beta = (\phi - 3/4)\kappa\delta^2/2\lambda, \qquad \lambda = \lambda \tag{10}$$

Then, as shown in Appendix A.6., the equations (2)–(5) of the representative model can be integrated to give the LQ equations (7) and (8). Appendix A.6. also shows that conditions for the LQ approximation to hold are that either the total dose or the dose rate be sufficiently small, specifically,

$$\text{either (A) } D \ll \frac{\alpha}{\beta}\frac{\phi - 3/4}{2(1-\phi)} \approx 17 \text{ Gy},$$
$$\text{or (B) } \dot{D} \ll \lambda\frac{\alpha}{\beta}\frac{\phi - 3/4}{2(1-\phi)} \approx 8 \text{ Gy/h} \tag{11}$$

Here the numerical values are rough, generic estimates, using the parameter values in the next subsection. The case of large doses with dose-rate small enough that equation (11B) holds must be treated with caution, since then irradiation time is typically so long that cell-cycle kinetic complications, neglected in all the models analysed here, can easily come into play.

The relation, equation (10), between the two models is of interest in both directions: the representative model supplies detailed molecular interpretations for the LQ formalism; and the LQ formalism covers the most important applications of this (and other) kinetic reaction-rate models. LQ equations can similarly be derived (Appendices A.6. and A.7.) from almost all common radiobiological reaction-rate models, including even models of saturable repair pathways rather than binary misrepair pathways. The form of the dose-protraction factor G is the same in all cases. Limitations on dose or dose-rate are needed, equation (11) being a typical example.

4.3. *Parameter values*

For detailed analyses specific parameter values are needed in the representative model. According to equation (10) three combinations of the four parameters can be obtained from the LQ parameters, α, β, and λ, which are comparatively well characterized because the LQ formalism has been applied so extensively. These parameters vary with cell type, cell-cycle kinetic status, and cell microenvironment, such as oxygenation status (e.g. Deschavanne *et al.* 1990, Steel 1991). Representative values for human cells are

$$\alpha = 0.3\ \mathrm{Gy}^{-1}, \qquad \beta = 0.05\ \mathrm{Gy}^{-2}, \qquad \lambda = 0.5\ \mathrm{h}^{-1} \tag{12}$$

Two-fold, or even larger, deviations from these values are not unusual for particular cell lines and/or particular experimental conditions (Deschavanne *et al.* 1990). For the fourth parameter it is convenient to focus on the number, δ, of relevant DSB produced per Gy. $\delta \approx 2$–$40\ \mathrm{Gy}^{-1}$ covers many detailed estimates obtained by using the RMR and similar models (e.g. Tobias *et al.* 1980, Tobias 1985, Curtis 1986, Sontag 1990, Hawkins 1996), often by comparisons to fixation time experiments (Appendix A.5.). A rough, generic estimate of δ, based specifically on the RMR model, is (Appendix A.3.):

$$\delta \approx 8\ \mathrm{Gy}^{-1} \tag{13}$$

The interpretation is that only a subset of the $\sim$40 DSB formed per Gy is relevant to survival (Subsection 3.1).

4.4. *Cellular radiosensitivity one-track (α) and two-track (β) action*

Subsection 4.2 gives a kinetic interpretation to the LQ formalism. In particular, equation (10) exemplifies a very general feature of ionizing radiation damage, that the LQ coefficient α corresponds to damage inflicted by individual radiation tracks, while β corresponds to damage inflicted by two different, independent radiation tracks.

Assuming the representative model, the fact that α corresponds to one-track action can be seen as follows. The coefficient δ which enters into α (equation (10)) corresponds to the production of DSB, with each DSB made by a single track, not by any cooperative action between tracks (see Subsection 3.1). The other factor in α, $1-\phi$, is the probability a restituted DSB undergoes lethal restitution (Subsection 3.1), a process independent of other DSB according to the model. Thus α refers to lethal lesions made by individual tracks. In general, other one-track lethality mechanisms contribute to α, and other models are also relevant. But in all cases α is associated with one-track mechanisms (Figure 2, Appendices A.1., A.6., and A.7.), not two-track mechanisms, and one-track mechanisms contribute only to α, not to β. Presumably the fact that cells have significantly different radiosensitivities at clinically relevant doses per fraction (Deschavanne *et al.* 1990, Steel 1991, West 1995) is due in large part to differences in one or more of these one-track mechanisms.

For the case of the representative model, the fact that β is associated with two-track action can be seen most easily from the fact that β is linearly proportional to κ equation (10), where κ is the rate coefficient for two-DSB binary misrepair (compare Figure 2 and Subsection 3.1). Two-DSB action corresponds to two-track action in the representative model because the Poisson assumptions of that model require that no track make more than one DSB (Appendix A.6.). Two-track action is also associated with β, rather than with α, in other binary misrepair models (Appendix A.6.) and in saturable repair models (Appendix A.7.); this association holds even at high LET, though the arguments then justifying it will not be given in the present review of low LET damage.

Equation (7) shows that α dominates the response to acute irradiation at very low doses (because D^2 is so small) and also the response to very low dose-rates (because G is so small; compare Figure 3C). The underlying reason is that in both cases only one-

track action is operative: at very low acute doses few cells have more than one DSB; and at very low dose rates, damage from any one track is almost fully repaired before the further damage from another track arrives. Thus (Steel 1991, Peacock *et al.* 1992) the low-dose response can be determined from the more readily measured low dose-rate response.

4.5. *High doses*

Most kinetic reaction rate models differ significantly from each other only for high doses or for endpoints determined before misrepair has run its full course. As an example of predictions for high doses, using the parameters in Subsection 4.3 and the explicit solution given in Appendix A.3., a curve for survival $S = \exp[-L(\infty)]$ as a function, $S(D)$, of acute dose can be plotted for the representative model of Section 3 (Figure 4). It is seen that for doses greater than about 5 Gy, the LQ approximation begins to deviate noticeably, consistent with the estimate in equation (11).

For sufficiently high acute doses, the model predicts a nearly linear, rather than a quadratic, dependence of $-\ln S$ on dose (Figure 4). Differentiating the explicit solution (Subsection A.3) shows that as D gets large, the survival curve slope $d \ln S/dD$ approaches the constant value $-\delta/4$. Almost all radiobiological reaction-rate models also show a nearly linear behaviour at large doses (Kiefer 1988a). For binary misrepair models, the intuitive reason is well known (Rossi and Zaider 1988, Brenner 1990). At sufficiently high doses, most DSB disappear via binary misrepair reactions, rather than via restitution. For example, for the representative model the rate κU^2 of DSB removal via binary misrepair in equation (2) dominates the rate $\lambda U(t)$ of DSB removal via restitution, rather than *vice-versa* (as can be seen by a counterpart of the argument in Appendix A.6.). But when binary misrepair dominates restitution, the number of lethal lesions produced is, approximately, some fixed fraction of the initial number $U(0)$ of DSB (e.g. is approximately $U(0)/4$ in the representative model of Section 3); since $U(0)$ is linear in dose (Section 2), average lethal lesion number is then approximately linear in dose.

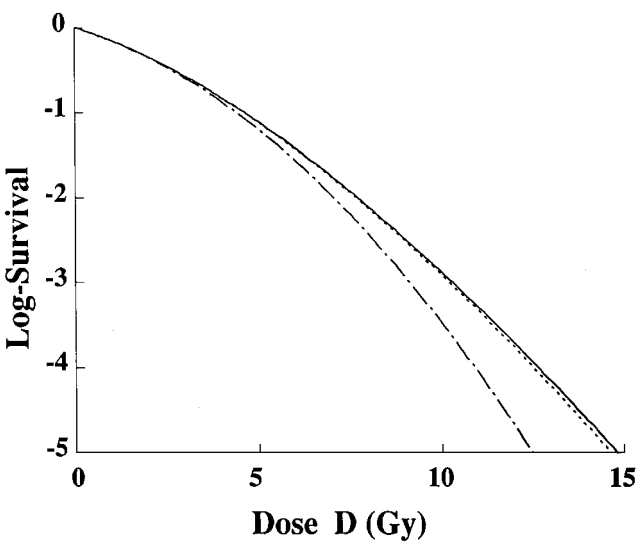

Figure 4. Dose-response curves. The solid line is an acute-dose survival curve for the representative RMR model of Subsection 3.1 with the parameters in equations (12) and (13). The lowest, dash-dot, line is the LQ approximation with α and β determined by the general relation, equation (10), between the parameters of the two models. At 5 Gy or less the differences are small. The same relation, equation (10), also gives a close correspondence between survival curves for arbitrary continuous low dose-rate irradiation and/or fractionation. The dotted line shows the very small corrections that are needed to the RMR curve if the Markov RMR model (Section B.7) is used to compute the number of cells without lethal lesions.

There is some experimental evidence, though not robust support, for high-dose linearity in survival data (e.g. Schneider and Whitmore 1963), chromosome aberration data (e.g. Lloyd and Edwards 1983, Simpson and Savage 1996), and pulsed field gel electrophoresis data on misrejoining of DNA fragments after very high doses (Lobrich *et al.* 1995).

The overall behaviour of the survival curve for acute irradiation given by typical kinetic reaction-rate models might thus be described as LQL, with a high dose nearly linear portion (Sachs and Brenner 1993, Radivoyevitch 1997). The change from near quadratic behaviour at intermediate doses to near linear behaviour at high doses is sometimes referred to as 'saturation' (Rossi and Zaider 1988), though that term is also used for different phenomena.

5. Dicentric chromosome aberrations

Radiobiological reaction-rate models are also used for endpoints other than survival, in particular the per-cell frequency of dicentric chromosome aberrations (Figure 1) produced during the G_0/G_1 phase of the cell cycle and assayed at the next metaphase. Such aberrations are of interest in connection with biodosimetry (Bauchinger 1995) and analysing the mechanisms of carcinogenesis (Hall 1994); they also provide direct information on the molecular mechanisms and kinetic pathways responsible for binary misrepair lethality (Figure 2). When the model of Section 3 is used for dicentrics, $L(\infty)$ specifies the dicentric frequency per cell. If cell-by-cell data is scored, rather than just per-cell averages, the dicentric endpoint probes the Poisson statistical distribution

(e.g. Lloyd *et al.* 1987) in a way cell survival assays cannot, for example by checking if the Poisson relation, equation (5) actually does hold for the experimental values of dicentric frequency $L(\infty)$ and the fraction, $S(\infty)$, of cells free of dicentrics.

5.1. *LQ approximation*

In most experiments the LQ formalism for average dicentric number,

$$L(\infty) = \alpha D + G\beta D^2 \qquad (14)$$

should be applicable (Subsection 4.2). A long series of observations on human peripheral blood lymphocytes (e.g. Lloyd and Edwards 1983, Edwards *et al.* 1996) have generally given, for low LET, roughly the parameter values

$$\alpha \approx 0.03\ \mathrm{Gy}^{-1}, \qquad \beta \approx 0.06\ \mathrm{Gy}^{-2}, \qquad \lambda \approx 0.5\ \mathrm{h}^{-1} \qquad (15)$$

with α larger for X-rays than for γ-rays. The estimates for β are more robust than those for λ or α, despite rather heroic efforts, scoring large numbers of cells, to characterize α (Lloyd *et al.* 1992, Bauchinger 1995), which dominates the response at sufficiently low doses, and is therefore important for applications to biodosimetry and to carcinogenesis risk estimation. Measurements for other human cell types generally give the same order of magnitude as the lymphocyte values in equation (15) (Cornforth and Bedford 1993). α is sometimes estimated not by scoring very large numbers of cells at low acute doses, but by applying continuous low dose-rate radiation (e.g. Pandita and Geard 1996), as discussed in Subsection 4.4.

Lethal DSB restitution, which contributes to α for cell killing (Subsection 4.4), does not produce dicentrics (Figures 1 and 2; Appendix A.1.). There are two main kinetic mechanisms which could produce the small but non-zero observed low LET value of α for dicentrics (equation (15)). The first possibility is that some dicentrics are created by a one-DSB kinetic pathway, whereby a single DSB can invade other chromatin not directly harmed by the radiation and then mimic a binary DSB interaction, making a dicentric (Goodhead *et al.* 1993). This mechanism can be quantified by using a non-zero value of $1-\phi$ for dicentrics in the representative reaction-rate model of Section 3. The second possible explanation is that dicentric-producing binary interactions may occur between DSB made by the same primary radiation track (Appendices A.9. and B.6.; compare Durante *et al.* 1996, Michalik and Frankenberg 1996, Moiseenko *et al.* 1996), perhaps with some kinetic advantage over interactions between DSB pairs made by different tracks (Greinert *et al.* 1995). The LPL model, Appendix A.4., can quantify such a one-track, two-DSB dicentric formation pathway.

Comparing equations (12) and (15), a striking fact is that β is rather similar for cell killing and for dicentric aberration production, as is λ, whereas the value of α is markedly smaller for dicentrics. In retrospect this pattern can be rationalized. Suppose most lethal binary misrepair consists of two-track dicentric chromosome aberrations (Figure 1) and rings, but there are also other one-track lethal molecular mechanisms (Figure 2, Appendix A.1.). Then the observed aberration/survival pattern of smaller α and approximately equal β. λ is exactly what one would expect (Subsection 4.4). It has often been suggested that chromosome aberrations may be the main contributors to cell lethality at low LET (e.g. Iliakis 1991, Schwartz 1992, Cornforth and Bedford 1993, Durante *et al.* 1995). Here it is being suggested that this relation applies mainly to the β (i.e. two-track) portion of the damage, with chromosome aberrations (including dicentrics, rings, and residual unrejoined breaks) being only one of several contributions to α (i.e. one-track) lethality.

5.2. *High doses*

At doses too high for the LQ approximation to be applicable, observations (Norman and Sasaki 1966) and a kinetic Monte-Carlo computer simulation model (Chen *et al.* 1996) show that the increase of dicentric frequency with dose is slower than would be predicted by the LQ formalism; eventually a levelling off and then a decrease occurs. This dose-response pattern is due to two effects: the saturation discussed in Subsection 4.5; and kinetic competition of dicentrics with other kinds of aberrations for a limited number of chromosome centromeres (i.e. 46 in a human cell).

6. Generalizations

The ideas in Sections 3–5 have been extended to analyse several additional aspects of radiation damage: multiple damage processing pathways operating simultaneously; a variety of other effects or endpoints; damage observed while repair and misrepair are underway; and cell-to-cell fluctuations in damage. A few recent results will now be reviewed.

6.1. *Multiple lesion types, pathways and rates*

There are many damage processing pathways that occur in a cell; each can have its own kinetics and dose-response relation. Evidence for multiple pathways comes from various directions: evidence that there are different types of DSB (Subsection 2.1);

bi-exponential or multi-exponential repair curves for excess chromosome fragments (e.g. Iliakis 1991, Foray *et al.* 1996, Greinert *et al.* 1995); analysis of pulsed field gel electrophoresis data (Radivoyevitch *et al.* 1997); and multiple repair times indicated by split dose or low dose-rate survival data (e.g. Nelson *et al.* 1989, Steel 1991, van Rongen *et al.* 1995).

From the molecular point of view, multiple or compound pathways are not surprising. For example, in Section 3, it could be more realistic to assume DSB free ends, rather than DSB, are the reactive units (Cornforth and Bedford 1993, Chen *et al.* 1996). Then a systematic kinetic theory would have to track averages for two different types of free ends: those whose partners have not yet participated in illegitimate reunions, so that restitution is still an option; and those which have in effect been divorced. Other plausible molecular scenarios also indicate multiple or compound pathways (Hahnfeldt *et al.* 1992, Radivoyevitch 1997).

A reaction-rate model for a multiple pathway can be constructed as follows. Suppose there are two kinds of DSB, each restituted with first order kinetics but with different time constants, each subject to lethal restitution, and each capable of binary misrepair, either independently or synergistically. Then, extending the arguments in Section 3 and in Appendix A.3., the rate equations would be

$$\left.\begin{aligned} \text{(A)}\ dU_i/dt &= \delta_i D - \lambda_i U_i - \kappa_i U_i^2 \\ &\quad - \kappa U_1 U_2 \qquad (i = 1, 2) \\ \text{(B)}\ dL/dt &= \sum_{i=1}^{2} [(1-\phi_i)\lambda_i U_i + (1-\psi_i)\kappa_i U_i^2] \\ &\quad + (1-\psi)\kappa U_1 U_2 \end{aligned}\right\} \tag{16}$$

where $\kappa = 0$ if there is no synergism.

If $\kappa_i U_i^2 + \kappa U_1 U_2 \ll \lambda_i U_i$ for $i = 1, 2$, a calculation very similar to that in Appendix A.6. shows that a 'two-time' LQ formalism is equivalent, at sufficiently low doses dose or dose-rates, to equation (16):

$$-\ln S = \alpha D + (G_1\beta_1 + G_2\beta_2) D^2 \tag{17}$$

Here G_1 and G_2 are generalized Lea-Catcheside dose-protraction factors with different repair rate constants, i.e.

$$G_i = (2/D^2) \int_{-\infty}^{\infty} D(t)\,dt \times \int_{-\infty}^{t} \exp[-\lambda_i(t-t')]D(t')\,dt' \qquad i = 1 \text{ or } 2 \tag{18}$$

The following parameter identifications are required

$$\alpha = \delta_1(1-\phi_1) + \delta_2(1-\phi_2);$$

$$\beta_i = \frac{(\phi_i - \psi_i)\kappa_i \delta_i^2}{2\lambda_i} + (\phi_1 + \phi_2 - 1 - \psi)\frac{\kappa\delta_1\delta_2}{\lambda_1 + \lambda_2}; \qquad i = 1, 2 \tag{19}$$

Evaluating total DSB number $U = U_1 + U_2$ after a single acute dose gives approximately bi-exponential decay, i.e.

$$U(t) = U_1(0)\exp[-\lambda_1 t] + U_2(0)\exp[-\lambda_2 t] \tag{20}$$

Equations (17) and (20) correspond to the experimental evidence discussed above for two repair times. There are other possible reaction-rate patterns for multiple or compound pathways, which have been discussed systematically by Hahnfeldt *et al.* (1992) and by Radivoyevitch (1997). Thus the experimental evidence for multiple pathways can be modelled by straightforward extensions of standard reaction-rate equations. In the LQ approximation the α terms typically add and the β terms reveal multiple repair times, as in equation (17).

6.2. *Some other generalizations*

There are many aspects of cellular response to radiation in addition to survival or chromosome aberrations for non-cycling cell populations. Aspects which have been analysed using kinetic reaction-rate equations include the following: proliferation during or after irradiation (e.g. Tucker and Travis 1990); cell-cycle redistribution effects (e.g. Yakovlev and Zorin 1988, Brenner *et al.* 1995, Hlatky *et al.* 1995, Hahnfeldt and Hlatky 1996); radiation-induced growth delays (e.g. Zaider *et al.* 1996); and the endpoints of cell transformation (e.g. Tobias *et al.* 1980) or mutation per surviving cell (e.g. Brenner *et al.* 1996).

6.3. *Assays at intermediate times*

One other way in which kinetic reaction-rate models are more general than the LQ formalism is that the latter really applies only to endpoints which are fully developed (Subsections 3.2 and 4.2). PCC (premature chromosome condensation) experiments (reviewed by Iliakis 1991, Cornforth and Bedford 1993) involve tracking lesions at intermediate times, during repair and misrepair. Then $U(t)$ in Section 3 is to be read as average number of breaks expressed

in the PCC assay and $L(t)$ is the average per-cell number of exchange-type chromosome abberations (such as those shown in Figures 1B and 1C). Assuming a PCC experiment where the dose is low enough that $\kappa U^2 \to 0$ is an adequate approximation in equation (2), and assuming a single acute dose given just before $t = 0$, equations (2) and (3) with $\phi = 1$ can be integrated to give

$$U(t) = U(0)e^{-\lambda t}, \qquad L(t) = U(0)^2\kappa(1 - e^{-2\lambda t})/8\lambda \tag{21}$$

The significant feature here is that the rate constant for L (which characterizes the gradual build-up of exchange-type aberrations) is 2λ, twice the DSB decay rate constant λ. Moiseenko *et al.* (1996) point out this difference in time constants; they discuss models which may explain why several data sets show a build-up of exchange-type aberrations at the same rate, or more slowly than, the decay of breaks (e.g. Durante *et al.* 1996, Evans *et al.* 1996, Greinert *et al.* 1996, Wu *et al.* 1996), contrary to equation (21).

Another relevant endpoint is measuring DNA fragment sizes after large doses, using pulse field gel electrophoresis (e.g. Friedl *et al.* 1995, Newman *et al.* 1997). The rejoining and misrejoining of radiation-produced DNA fragments can be tracked in time (Lobrich *et al.* 1995), and modelled by a variant of the kinetic reaction-rate equations in Section 3 (Radiovoyevitch 1997).

6.4. *Markov models for cell-to-cell damage fluctuations*

In kinetic reaction-rate models, Poisson distributions for lesions are usually assumed (e.g. Subsection 3.1). At high LET the distribution of lesions is not governed by Poisson statistics, and even at low LET some deviations could occur (Harder 1988, Albright 1989). Analysing what intercellular distributions do hold involves combining microdosimetry (Kellerer 1985, Goodhead 1987, Rossi and Zaider 1996) with stochastic chemical kinetics (Erdi and Toth 1989). One obtains continuous-time Markov chain models (e.g. Hug and Kellerer 1966, Curtis 1988, Albright 1989), which track the time development of all individual probabilities (for example the probability a cell has exactly ν relevant DSB for $\nu = 1, 2, \ldots$). There are infinitely many unknown functions, with one differential equation for each. Standard techniques (Erdi and Toth 1989, Sachs *et al.* 1992) often allow explicit integration of all the equations, and numerical integration to high accuracy is almost always feasible. For example, Appendices B.6. and B.7. estimate by how much low LET lesion distributions can deviate from Poisson distributions. Figures 4 and 5 show that the influence of such deviations on typical low LET data is minor.

For a fully developed endpoint (Subsection 3.2) and a single acute dose, continuous-time Markov chains are equivalent to discrete-time Markov chains (Sachs *et al.* 1992). The essence of such a discrete-time chain is that damage infliction and processing are viewed sequentially, in steps. This way of analysing damage processing is quite useful (Appendix B.7.; Albright and Tobias 1985, Goodwin and Cornforth 1991, Hlatky *et al.* 1991) and, contrary to what one might expect, readily yields numerical or analytical results (Hahnfeldt *et al.* 1992, Radivoyevitch 1997).

The most powerful way of implementing Markov, sequential, probabilistic calculations of damage processing is to use Monte-Carlo computer simulations: whenever a particular cell has to make a probabilistic choice, the computer rolls the appropriate dice. Many cells are simulated and the results then averaged. Monte-Carlo simulation models are widely applicable in many areas (Ripley 1987). They are extremely flexible, and usually all sorts of additional effects can be incorporated without changes in a basic approach, the main (and quite serious) danger being overparametrization.

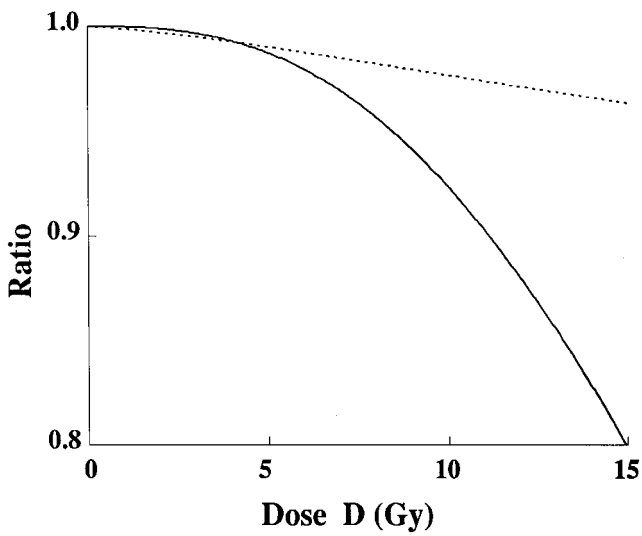

Figure 5. Stochastic effects. The solid curve shows, for the Markov version of the model in Section 3 and the same parameters as in Figure 4, the quantity $Z/\exp[-L(\infty)]$, where Z is the fraction of cells without lethal lesions at large times. The dotted curve shows the cell-to-cell variance of lethal lesions divided by the mean number of such lesions. Both quantities would be 1.0 if the cell-to-cell distribution of lethal lesions were Poisson. Only small deviations from Poisson behaviour occur, even at large doses.

6.5. *Kinetics and proximity effects*

One aspect of radiobiological damage processing kinetics which calls for the full power of Monte-Carlo computer simulations is spatial inhomogeneity. Equations like (2) tacitly assume that the initial spatial separation of two DSB does not influence their probability of undergoing binary misrepair, but it is known that, on the contrary, 'proximity' effects are important, i.e. if two DSB are formed far apart, their probability of undergoing binary misrepair is greatly reduced due to diffusion limitations (reviews in Savage 1996, Sachs *et al.* 1997). Simple reaction-rate models do not incorporate proximity effects. Calculations using sites (Appendix A.8.) and non-kinetic calculations (Appendix A.9.) allow some estimates, but Monte-Carlo computer simulations are apparently essential for any detailed analysis. Such simulations have long been used for studying post-irradiation kinetics on very short time scales (Varma and Chatterjee 1994). More recently, they have been applied to the kinetics, on time scales of a minute or more, discussed in the review. For example, Brenner (1990) and Edwards *et al.* (1996) have considered binary DSB interactions modulated by proximity effects. Chen *et al.* (1996, 1997) use sites (Appendix A.8.) to incorporate chromosome localization and proximity effects, combined with Monte-Carlo simulations to track the post-irradiation formation of many different kinds of simple or complex chromosome aberrations, involving particular chromosomes of specified lengths.

7. Summary

The kinetics of low-LET damage production, repair, and misrepair have been discussed. A major kinetic pathway involves restitution of DSB competing with binary misrepair of DSB (Sections 2 and 3). other mechanisms, such as direct infliction of lethal lesions, are also important for cell killing (Appendix A.1.). Each pathway can have its own kinetics, and specific kinetic reaction-rate models have been developed for particular pathways (Section 3 and Appendices A.3.–A.7.). Such kinetic reaction-rate models lead to predictions for survival, as a function of the dose and of the time-pattern of dose delivery (Section 4). The kinetic models can also be applied to chromosome aberrations or various other endpoints (Sections 5 and 6), and can be extended to highly flexible computer-based models (Section 6).

8. Discussion

Ionizing radiation damages cells by many different molecular mechanisms. This review analysed reaction rates for various damage pathways. The kinetics of such pathways can be linked to dose-response relations for measurable endpoints, and this linkage allows a mechanistic interpretation of measured dose-response parameters. Kinetic models also relate response to an acute dose with response to a protracted dose, spread out temporally in any way.

It was shown that, for low or intermediate doses, almost all of the kinetic reaction-rate models predict an LQ dose-response relation, with the standard (generalized Lea-Catcheside) dependence on dose protraction. In fact these models predict LQ behaviour even at high doses if dose delivery is sufficiently protracted, though in this case cell-cycle kinetic effects could require modifications. If more than one mechanism is operative, a sum of LQ terms is expected, and there is some experimental evidence for such compound behaviour. Thus modelling of the underlying kinetics suggests the LQ formalism is likely to be appropriate, regardless of the fact that there is still an incomplete picture of how cells process ionizing radiation damage.

The kinetic reaction-rate models supply mechanistic interpretations for the LQ parameters α and β. It was argued that β is similar for dicentrics and cell survival, corresponding to a scenario in which two-track lethal lesions are predominantly exchange-type chromosome aberrations, such as dicentrics and rings. On the other hand, one-track (i.e. linear, dose-rate-independent, α) lethality probably results mainly from other mechanisms, such as small deletions, residual chromosome breaks, or apoptosis.

Like the modelling results described here, experimental results over the last decade tend to validate the LQ formalism as far as applications to low and intermediate doses are concerned. No doubt some caution in accepting the LQ formalism as generally applicable is needed. For example, if the dominant influence on survival is inducible repair (Shadley *et al.* 1987), or delayed death (Hendry and West 1995), or genomic instability (Morgan and Murnane 1995), LQ behaviour would not necessarily be expected to hold, at least not with the standard dependence on dose-protraction, and these possibilities have not been robustly excluded. But the theoretical and experimental evidence for LQ behaviour at low or moderate doses is, on balance, strong.

Two important applications of the LQ formalism at very low doses are biodosimetry and extrapolation of radiation-induced cancer risk estimates to very low doses. For biodosimetry, where exchange-type aberrations such as dicentrics or translocations are often used to reconstruct past exposures, the α coefficient is usually what is required. The α coefficient for these endpoints is small and hard to measure.

The theoretical considerations that have been given support the idea that the LQ formalism is applicable and the relevant α coefficient can be estimated by using low dose rates, in addition to or instead of low acute doses.

For extrapolation of radiation-induced cancer risks to very low doses, the situation is more complex in that while haematopoietic cancers (leukemias and lymphomas) are typically assoicated with exchange-type aberrations such as translocations (Nowell 1997), solid tumours are most often associated with smaller-scale damage, such as small deletions (Le Beau and Rowley 1986, Rabbits 1994). Thus while haematopoietic cancers might be expected to exhibit an LQ dose-response relationship, solid tumours might be expected to exhibit a more linear behaviour; though not universal, this pattern often does hold, both in the human studies at Hiroshima and Nagasaki (Pierce *et al.* 1996), and in animal studies (e.g. Upton *et al.* 1970, Shellabarger *et al.* 1986). For both groups of cancer, extrapolation to low doses would require estimation of the α term, but this may be much easier for solid cancers than for haematopoietic cancers, and fractionated or low dose rate exposures could again be advantageous for the latter estimations.

At the intermediate doses of $\approx$2 Gy relevant to conventional fractionated radiotherapy, the LQ formalism, by reference to the underlying kinetic models, should be applicable. At considerably higher acute doses, a predominantly linear reponse of log-survival is predicted by most kinetic reaction-rate models, and thus the LQ equation begins to fail. However, insights obtained through analysing underlying kinetics as to why the LQ formalism fails—through saturation effects—may allow appropriate modifications to be made.

Can one do better? Are there kinetic models appropriate to single acute doses as high as those used, for example, in stereotactic radiosurgery? Can modelling cope with the cell-cycle kinetic complications that arise in many situations, for example in analysing brachytherapy? Further progress on quantitative kinetic modelling is possible, and seems to be needed. Purely phenomenological or statistical approaches to dose-response relations and dose-protraction effects have, in our opinion, gone about as far as they can go. Identification of damage pathways on the molecular level will be increasingly important. However, qualitative molecular investigations, despite their current popularity, are not likely to be very useful either. The question is not whether a given gene product has some effect or shows some response to radiation; the question is what damage pathways are *dominant* for the important biological endpoints. That is a question which requires quantification, using kinetic models of the kind discussed here.

For now, these models supply a useful connection between molecular mechanisms and the LQ formalism. Despite all the uncertainties and limitations involved, this connection is, we would suggest, a triumph of radiobiology and an appropriate tribute to the genius of Douglas Lea.

Acknowledgements

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Appendix A. Radiobiological reaction-rate models and their interrelations

A.1. *Kinetic reaction-rate models*

Appendix A describes, interrelates, and relates to the LQ model additional models which track average lesion numbers using the differential equation methods of chemical kinetics: the RMR model (Tobias *et al.* 1985), the LPL model (Curtis 1986), a saturable repair model (Kiefer 1988b), and many variants of these models. Such mechanistic reaction-rate models have a long history (Steel 1996). Two conference reports (Kiefer 1988a, Chadwick *et al.* 1992) give a fairly comprehensive historical over-view. Recent comparisons of models have been given by Sontag (1990), Zackrisson (1992), Kiefer (1993), Fertil *et al.* (1994), and Hanin *et al.* (1994).

The main reason for the plethora of models is that there are many damage mechanisms in addition to the scenario, of viable or lethal DSB restitution competing with lethal or viable binary DSB misrepair, that motivates the representative radiobiological reaction-rate model of Section 3. For direct one-track action as in Figure 2E, there are many possible lethal outcomes, including the following: point mutations; small deletions without a DSB as intermediate state (Curtis 1986, Hagen 1989); damage leading to apoptosis (Dewey *et al.* 1995, Meyn *et al.* 1996); DSB which neither restitute nor undergo binary misrepair and therefore remain as 'residual DSB' (Iliakis 1991, Steel 1991, Cornforth and Bedford 1993, Obaturov *et al.* 1993, Savage 1995); additional lethal lesions caused by damage fixation (Appendix A.5.); perhaps DNA-protein crosslinks, base-damage or single strand breaks (Frankenberg-Schwager 1989, Hagen 1989); perhaps lesions generated by a single DSB invading undamaged DNA and mimicking binary misrepair

(Goodhead *et al.* 1993); and damage involving binary misrepair of DSB produced by a single track, as is important at high LET (Appendix B.6., Kellerer 1985), perhaps for soft X-rays, and perhaps even for gamma-rays or hard X-rays (Edwards *et al.* 1996, Greinert *et al.* 1996, Michalik and Frankenberg 1996). Complex chromosome aberrations, involving more than two DSB, can contribute to lethality (Savage 1995). It has been suggested (Preston 1990) that binary misrepair could involve damaged bases rather than DSB. It has sometimes been argued that perhaps DSB are the only kind of lesions which lead to significant lethality (review in Pfeiffer *et al.* 1996), but even if this is true, there appear to be a number of different pathways involved.

The basic approach of the present review is to regard various models as appropriate for different kinetic pathways, with some pathways dominant while others are minor, and to emphasize that almost all pathways considered correspond to the LQ formalism at low and intermediate doses. Many papers do not share these perspectives.

A.2. *Dose rates*

To get general results about models, it is useful to work with an arbitrary time-varying dose-rate function $\dot{D}(t)$. Then the total dose is the integral:

$$D = \int_{-\infty}^{\infty} \dot{D}(t)\,dt \qquad \text{(A.1)}$$

where the lower limit could alternatively be taken as any time before irradiation starts. Two simple examples of $\dot{D}$ are: (A) an acute dose D delivered at time t_0, by a source operating at a high dose rate ρ; or (B) irradiation with total dose D, starting at $t = 0$, by an exponentially decaying radioactive source with time constant $k = \ln 2/T$, where T is the half-life. In both cases the dose rate function $\dot{D}(t)$ is different from zero only for certain time intervals, as follows:

$$\begin{aligned} &\text{(A)}\ \dot{D}(t) = \rho \quad \text{for } t_0 - D/2\rho \leqslant t \leqslant t_0 + D/2\rho; \\ &\text{(B)}\ \dot{D}(t) = kDe^{-kt} \quad \text{for } t \geqslant 0 \end{aligned} \qquad \text{(A.2)}$$

More complicated regimens are described by more complicated functions D. In equation (A.2A) irradiation time D/ρ is often much shorter than any repair time or misrepair time of interest; then it is permissible and often convenient to use the formal limit as ρ becomes infinite (acute irradiation).

A.3. *The RMR model*

In the RMR model the term 'uncommitted lesions' is used for lesions whose per-cell average is $U(t)$; these are presumably (some subset of) DSB. The rate equations for the model are (Tobias 1985)

$$\begin{aligned} &\text{(A)}\ dU/dt = \delta\dot{D} - \lambda U - \kappa U^2; \\ &\text{(B)}\ dL/dt = (1-\phi)\lambda U + (1-\psi)\kappa U^2 \end{aligned} \qquad \text{(A.3)}$$

Equation (A.3) was motivated in Subsection 3.1, except that in (A.3B) the factor $1 - \psi$ replaces (1/4). $1 - \psi$ is interpreted as the average number of lethal lesions made for every uncommitted lesion that disappears via binary misrepair.

Exact analytic solutions of rate equations can often be found when the dose rate $\dot{D}$ is zero or has some special form. As an example, here are the solutions (Tobias *et al.* 1980) for the RMR equation (A.3), assuming a single acute dose just before $t = 0$ so that $\dot{D} = 0$ for $t \geqslant 0$.

$$\begin{aligned} &U(t) = [U(0)/f]e^{-\lambda t}, \\ &L(t) = (\psi - \phi)(\lambda/\kappa)\ln f \\ &\qquad + (1-\psi)[U(0) - U(t)] \end{aligned} \qquad \text{(A.4)}$$

$$\text{where } t \geqslant 0, \qquad U(0) = \delta D,$$

$$\text{and } f = f(t) = 1 + [\kappa U(0)/\lambda](1 - e^{-\lambda t})$$

For $t \to \infty$, λ and κ appear only in the ratio κ/λ, not separately. This reduction of parameter numbers illustrates the principle of repair ratios (Hlatky *et al.* 1991), that only ratios of repair rate constants, not the constants themselves, influence the response at long times after one acute dose. Explicit solutions like equation (A.4) have only a limited usefulness. It is often easier and clearer to work with the differential equations, using numerical integration if numerical results are needed, and manipulating the equations themselves in conceptual arguments (e.g. the argument of the next paragraph).

A simple rescaling can be used to relate the solutions of the RMR model with $\psi = 0$, a value which is often assumed but is not realistic as regards molecular interpretations (Section 3), to solutions of the model with $\psi = 3/4$, i.e. of the representative model in Section 3. By comparing the rate equations one shows that the two cases for ψ can be transformed into each other as follows.

$$\begin{aligned} &L \to L, \qquad U \to 4U, \qquad \phi \to \phi, \\ &\lambda \to \lambda, \qquad \kappa \to \kappa/4, \qquad \delta \to 4\delta, \\ &\psi = 0 \to \psi = 3/4 \end{aligned} \qquad \text{(A.5)}$$

The interpretation then changes because, for example, U is not DSB number if $4U$ is DSB number. But equation (A.5) can be used to generate the mathematical solutions of one case with any initial conditions and any dose-rate function, from the solutions

of the other case, and used to transfer empirical parameter estimates between the two cases. The practical effect of these rescalings for this review is that the rescaling $\delta \to 4\delta$ brings the δ estimates obtained by comparing the RMR model to various experiments (Tobias 1985) up to approximately $\delta \geqslant 8\ \mathrm{Gy}^{-1}$, the number used in Section 4.

A.4. *The LPL model*

The LPL model deals with 'potentially lethal' lesions, having average number $n_{PL}(t)$ and with lethal lesions, whose average number is denoted by $n_L(t)$. The model's reaction-rate equations are (Curtis 1986):

$$\begin{aligned} &(\mathrm{A})\ dn_{PL}/dt = \delta D - \lambda n_{PL} - \kappa n_{PL}^2, \\ &(\mathrm{B})\ dn_L/dt \ = \alpha \dot{D} + c\kappa n_{PL}^2 \quad \text{where } c = 1 \end{aligned} \tag{A.6}$$

For the reasons discussed in Section 3, the model obtained by setting $c = 1/4$ (rather than 1) is probably more realistic; it will be discussed later. The really distinctive features in equation (A.6), are the presence in (B) of an $\alpha\dot{D}$ term and the absence in (B) of a term linearly proportional to n_{PL}. The αD term in the LPL equation (A.6B), depending directly on dose, is appropriate for a directly lethal pathway such as that shown in Figure 2E; but the LPL model does not model lethal restitutions, such as those shown in Figure 2B. Conversely, the RMR model is appropriate for the lethal restitution pathway Figure 2B but not for the directly lethal pathway Figure 2E. Actually both pathways are occurring in the cell, which motivates introducing models which combine the RMR and LPL models (Obaturov *et al.* 1993, Hawkins 1996). Unfortunately, the number of adjustable parameters then increases.

Intuitively, lethal restitution (e.g. Figure 2B) and direct creation of lethal lesions (Figure 2E) seem similar and this similarity is reflected in the mathematics: by a mathematical trick any solution of the LPL rate equations, for any dose rate function D, can be obtained from a corresponding solution of the RMR model. Specifically the following theorem, due to N. Albright (private communication 1991), holds. Let n_{PL}, n_L be a solution of the LPL rate equations (A.6). Set

$$\begin{aligned} &U = n_{PL}, \qquad L = n_L - (\alpha/\delta) n_{PL}, \\ &\psi = 1 - [c + (\alpha/\delta)], \\ &\text{and } \phi = 1 - (\alpha/\delta) \end{aligned} \tag{A.7}$$

leaving δ, κ, and λ unchanged. Then U, L is a solution of the RMR rate equations. The proof consists of plugging the substitution (A.7) into equation (A.3). Albright's theorem does not mean the two models are identical; it does mean that survival curves cannot easily distinguish between one-track direct lethality (LPL, e.g. Figure 2E) and one-track lethal restitution (RMR, e.g. Figure 2B) pathways.

In analysing the solutions of the LPL or other reaction-rate models it is often convenient to think of acute doses, given by themselves or perhaps during a continuous low dose-rate regimen, as simply changing the values of quantities like n_{PL}, n_L instantaneously. For the LPL model one has the following rule for how much lesion averages jump when an acute dose D is applied.

$$\begin{aligned} &(\mathrm{A})\ \Delta n_{PL} \equiv n_{PL}(\text{just after}) - n_{PL}(\text{just before}) \\ &\qquad = \delta D, \\ &(\mathrm{B})\ \Delta n_L = \alpha D \end{aligned} \tag{A.8}$$

This result can be proved in various ways. For example, inserting the expression (A.1A) for D into (A.6A), integrating (A.6A) over the short time interval D/ρ, taking the limit $\rho \to \infty$ gives (A.8A). Similar results, e.g. equation (4) hold for other models.

According to the arguments of Section 3, it would be more realistic to modify the LPL model by choosing $c = 1/4$ in equation (A.6) rather than using $c = 1$ (i.e. on average four potentially lethal lesions disappear for every lethal lesion made by lethal binary misrepair). Much as in equation (A.5), the two cases can be transformed into each other by rescaling as follows.

$$\begin{aligned} &n_L \to n_L, \qquad n_{PL} \to 4n_{PL}, \qquad \alpha \to \alpha, \\ &\lambda \to \lambda, \kappa \to \kappa/4, \qquad \delta \to 4\delta, \\ &c = 1 \to c = 1/4 \end{aligned} \tag{A.9}$$

A.5. *Damage fixation times*

Additional lethal damage formation (e.g. making DSB into lethal lesions), sometimes occurs via damage fixation at some specific time prior to the completion of damage processing, the specific time being dictated by cell cycle kinetics or by the experimental assay. Then the endpoint of interest is not fully developed (Subsection 3.2). For example replating cells shortly after irradiation can involve damage fixation (Iliakis 1991). Most kinetic reaction-rate models have provisions for a damage fixation mechanism. For example consider an acute dose given just before $t = 0$. Then in the RMR model it is assumed that at some later time t_r, set by the cell and regarded as an adjustable parameter, all the remaining 'uncommitted lesions' become lethal, i.e. the surviving fraction is given by Poisson statistics (Appendices B.1 and B.5) as

$$S = \exp[-L(t_r) - U(t_r)] \tag{A.10}$$

For $t_r \gg 1/\lambda$, $U \to 0$, so equation (A.10) becomes

the relation $S = \exp[-L(\infty)]$ already discussed in Section 3, and the extra adjustable parameter is not needed. Many other models analyse damage fixation similarly (e.g. Curtis 1986, Kiefer 1988b, Sontag 1990, Obaturov *et al.* 1993, Ostashevsky 1993, Hawkins 1996). How models which assume damage fixation at a time set by the cell itself apply to protracted dose delivery regimens is often not specified.

A.6. *The LQ formalism as an approximation to binary misrepair models*

Next the LQ formalism is derived, including the form, equation (8), of the generalized Lea-Catcheside dose-protraction factor, as an approximation to the LPL model, assuming low or intermediate doses and a fully developed endpoint. Related arguments have been given by Lea (1946), Thames (1985), Curtis (1986), Thames and Hendry (1987), Obaturov *et al.* (1993), Hanin *et al.* (1994), and Hawkins (1996). The specific claim here is the following. Suppose the term $\kappa\eta_{PL}^2$ is negligible in equation (A.6A) (but not necessarily in (A.6B)). Then $L(\infty)$ is given by the LQ equation (7), with the generalized Lea Catcheside factor G duly given for any kind of dose-protraction by equation (8), and with the following identification of the parameters:

$$\alpha = \alpha, \qquad \beta = c\kappa\delta^2/2\lambda, \qquad \lambda = \lambda \tag{A.11}$$

The proof starts by integrating equation (A.6A) with $\kappa\eta_{PL}^2 \to 0$ to get (Boyce and Diprima 1997)

$$\eta_{PL}(t) = \delta e^{-\lambda t} g(t), \qquad \text{where } g(t) = \int_{-\infty}^{t} D(t') e^{\lambda t'} dt' \tag{A.12}$$

Next, integration by parts and using the integral (A.2) for total dose D gives the following auxiliary formulae for $g(t)$ in equation (A.12):

$$\int_{-\infty}^{\infty} dt e^{-\lambda t} g(t) = \frac{D}{\lambda},$$

$$\text{and} \int_{-\infty}^{\infty} dt e^{-2\lambda t} g^2(t) = \frac{1}{\lambda}\int_{-\infty}^{\infty} dt e^{-\lambda t} g(t)\dot{D}(t) = \frac{D^2}{2\lambda} G \tag{A.13}$$

where G is the generalized Lea-Catcheside double integral, equation (8). Inserting (A.12) into (A.6B), integrating, and using (A.13) gives

$$\eta_L(\infty) = \alpha D + c\kappa\delta^2 \int_{-\infty}^{\infty} dt e^{-2\lambda t} g^2(t) = \alpha D + (c\kappa\delta^2/2\lambda) D^2 G \tag{A.14}$$

Using the relations (A.11) for the parameters, and comparing equation (A.14) with (8) shows the theorem is true for the LPL model.

Now the RMR model will be considered. Substituting Albright's relation, equation (A.7), between the LPL and RMR models, into equation (A.14), shows that a corresponding LQ approximation, based on $\kappa U^2 \ll \lambda U$, holds for the RMR model, with the following identification of parameters:

$$\alpha = (1-\phi)\delta, \qquad \beta = (\phi - \psi)\kappa\delta^2/2\lambda, \qquad \lambda = \lambda \tag{A.15}$$

Assuming a single acute dose, the condition $\kappa U^2 \ll \lambda U$ used above is a low/intermediate dose approximation. This fact can be seen from the following argument, which is essentially due to Lea (1946, p. 263). Assume the single acute dose D is given just before $t = 0$. Then $U(0) = \delta D$ (compare equation (4)), so at $t = 0$ a comparison in equation (A.3A) between the rate of DSB removal by restitution, $\lambda U(0) = \lambda\delta D$, and by binary misrepair, $\kappa U^2(0) = \kappa(\delta D)^2$, strongly favours restitution whenever

$$\lambda\delta D \gg \kappa(\delta D)^2, \qquad \text{i.e. } D \ll \frac{\lambda}{\kappa\delta} = \frac{\alpha}{\beta}\frac{\phi-\psi}{2(1-\phi)} \tag{A.16}$$

If equation (A.16) holds, then, at times later than $t = 0$, as DSB are removed by restitution and misrepair, the discrepancy between $\lambda U(t)$ and $\kappa U^2(t)$ becomes still greater. Thus, for acute doses which obey (A.16), $kU^2 \ll \lambda U$ at all times, and the discussion above shows that this approximation leads to the LQ formalism. A corresponding argument shows that, for prolonged irradiation of any kind, and LQ formalism with the parameter identification (A.15) is valid if the dose rate is low enough. A sufficient condition is

$$\dot{D} \ll \lambda \frac{\alpha}{\beta}\frac{\phi-\psi}{2(1-\phi)} \tag{A.17}$$

The ratio, α/β, is often characterized more accurately than α or β separately, mainly because this ratio plays a key role in iso-effect calculations (Thames and Hendry 1987). Equations (A.16) and (A.17) with $1-\phi \ll 1$, $\psi \approx 3/4$ show that whenever dose D is no larger than $\approx\alpha/\beta$, or dose-rate $\dot{D}$ is no larger than $\approx\lambda\alpha/\beta$, one is well within the range where the LQ approximation to the RMR model is applicable.

As a cross check on the mathematical manipulations, setting $t = \infty$ in the explicit special case (A.4) for one acute dose and expanding as a power

series in the dose gives from the first two terms the same parameter identification for α and β as does the general argument applicable to arbitrary dose-rate functions, namely equation (A.15).

In an overall sense, the RMR acute dose survival curve (Figure 4) is similar to its LQ approximation. This can be seen by a useful two-parameter characterization of survival curves, which takes into account their behaviour at all doses, not just at small doses. The characterization is given by the mean $\bar{D}$ and variance σ^2 for the probability density $p(D)$ defined by $p(D) = -dS(D)/dD$; $\bar{D}$ is called the 'mean inactivation dose' and $RS = \bar{D}^2/\sigma^2$ is called the 'relative steepness' (Hug and Kellerer 1966, Rossi and Zaider 1996). Using numerical methods to compute mean inactivation dose and relative steepness for the RMR curve and its LQ approximation in Figure 4 gives $\bar{D}_{RMR} = 2.21$ Gy, $\bar{D}_{LQ} = 2.13$ Gy, $RS_{RMR} = 1.58$, and $RS_{LQ} = 1.70$. The fact that the RMR and LQ values are within less than 10% of each other indicates that, taking all doses into account, the RMR curve and its LQ approximation are similar.

By using first-order non-singular perturbation theory (Brenner *et al.* 1997) one can show that other binary misrepair kinetic models, such as the compound model of Subsection 6.1, also have LQ behaviour (compare Obaturov *et al.* 1993, Hawkins 1996). As will be discussed next, models which use a quite different approach also lead to the LQ formalism, including the same generalized Lea-Catcheside dose-prolongation factor G.

A.7. *A saturable repair model*

Various saturable repair models have been introduced (e.g. Haynes 1964, Reddy *et al.* 1990, Sanchez-Reyes 1992), though the molecular interpretations, biological consequences, and mathematical implications of such models have not been worked out as thoroughly as in the case of binary misrepair models. The saturable repair model of Kiefer (1988b) is reasonably typical. It can be written in terms of two rate equations, for the average number $N(t)$ of 'initial lesions' and average number $L(t)$ of lethal lesions:

$$\text{(A)}\ \frac{dN}{dt} = \delta D - \frac{\lambda_1 N}{1+\varepsilon_1 N} - \frac{\lambda_2 N}{1+\varepsilon_2 N}; \qquad \text{(B)}\ \frac{dL}{dt} = \frac{\lambda_2 N}{1+\varepsilon_2 N} \tag{A.18}$$

λ_i and ε_i, $i = 1, 2$, are adjustable parameters. The term $\varepsilon_1 N$ corresponds to saturable repair (specifically, Michaelis-Menten) kinetics: as N gets large, the repair rate $\lambda_1/(1+\varepsilon_1 N)$ per unit initial lesion, which is λ_1 when $\varepsilon_1 N \ll 1$, becomes smaller, corresponding to saturating the repair system. Similar comments apply to the misrepair term involving λ_2 and ε_2. The model gives rise to shouldered survival curves if $\varepsilon_1 > \varepsilon_2$ (Kiefer 1988b). Using non-singular first order perturbation theory it can be shown (Brenner *et al.* 1997) that equation (A.18) also leads to the LQ formalism, including the generalized Lea-Catcheside dose-protraction factor, in an appropriate approximation. The following parameter identifications are required:

$$\lambda = \lambda_1 + \lambda_2, \qquad \alpha = \lambda_2 \delta/\lambda, \qquad \beta = \delta^2 \lambda_2 \lambda_1 (\varepsilon_1 - \varepsilon_2)/2\lambda^2 \tag{A.19}$$

For the special case of a single acute dose, this result was previously obtained by Kiefer and Lobrich (1992).

Sublesions in the Kiefer saturable repair model are not repaired with first order kinetics and do not interact directly in a reaction like lethal binary misrepair. But there is an indirect interaction: one sublesion uses the enzyme another needs. For low and intermediate doses, as has just been proved, the indirect interaction mimics lethal binary misrepair competing with non-saturable repair as far as leading to LQ behaviour is concerned, including even the details of cell sparing by dose-protraction. The fact that both binary misrepair and saturated repair lead at low or intermediate doses to the same, LQ, formalism for any type of dose protraction partially explains the otherwise somewhat puzzling similarity (Goodhead 1987) between the consequences of the two mechanisms.

For other saturable repair models (e.g. Sontag 1990) a wholly similar theorem can be proved. An exception is Goodhead's 'suicide enzyme' model (Goodhead 1985), which does not seem to have the LQ formalism as an approximation, except in the trivial sense of a power series for response to a single acute dose, the reason being that at low doses Goodhead's model is based on damage fixation occurring before repair and misrepair have run their full course (so that the limit $t \to \infty$ is not applicable).

A.8. *Site models*

The simplest way to incorporate proximity effects (Subsection 6.5) into radiobiological reaction-rate models is to partition the cell nucleus into interaction sites, with binary misrepair allowed only for DSB formed within the same site (e.g. Chen *et al.* 1996, Hawkins 1996, Savage 1996, Radivoyevitch *et al.* 1997). For per-cell averages of lesions whose fluctuations are governed by Poisson statistics, assuming more than one site makes little difference. For example, if in each site kinetics are governed by the model of

Section 3, then by simply adding the rate equations one shows that the total number of DSB or lethal lesions, as sums of contributions from each site, obey rate equations of exactly the same form, with some obvious rescalings of the parameters. Closer consideration of equation (5) shows that the validity of this trick depends on the assumption that DSB and lethal lesion numbers in different sites are independent of each other, with the intersite fluctuations governed by Poisson statistics (so that the totals are also Poisson, by the theorem in Appendix B.4 below). In any situation where there are significant deviations from Poisson statistics, as can occur for various reasons (Appendices B.6 and B.7), the site number has a significant influence (Harder 1988, Brenner and Sachs 1994).

A.9. *Non-kinetic models for dose-response relations*

This review emphasized kinetics. A kinetic approach is clearly indicated for doses which are protracted in time and for endpoints which are not fully developed. But kinetic models are informative in any case. It is often more useful to consider an acute dose arriving in small increments, and to consider the biological response gradually developing after irradiation, than to try to jump directly from total acute dose to final fully developed damage.

Multi-target, multi-hit models and some of their generalizations (Hanin *et al.* 1994) do make such a jump. So does the Theory of Dual Radiation Action. This theory can be used for protracted irradiation regimens (Kellerer and Rossi 1972), and is then very similar to the LQ formalism, but it avoids detailed kinetic equations. The resulting simplification often enables more careful consideration of spatial inhomogeneities within the cell nucleus (Subsection 6.5) than is readily possible in kinetic reaction-rate models (e.g. Kellerer and Rossi 1978, Brenner *et al.* 1994).

The Theory of Dual Radiation Interaction assumes that the dominant contribution to α in the LQ formalism is the formation of 'sublesion' pairs by a single event (i.e. that α is due to one-track binary misrepair), which implies a relation between α and β microdosimetrically (Kellerer 1985). However, at low LET, unless proximity effects are very important, this microdosimetric contribution to α is too small to account for the observed values (Goodhead 1987). The radiobiological reaction-rate models discussed in this review all assume that there are also other one-track lethality mechanisms, e.g. pathways B or E in Figure 2, which can make additional contributions to α.

Appendix B. The Poisson distribution

B.1. *Definition of the Poisson distribution*

The Poisson distribution describes whole-number fluctuations. It is used for many different radiobiological quantities: the number of cells per tumour; the number of radiation tracks per cell; the number of DSB per chromosome arm; etc. Because the Poisson distribution is virtually ubiquitous it is important to distinguish its various uses; for example, at high LET it is usually reasonable to suppose that the number of radiation tracks per cell nucleus is Poisson-distributed, but often unreasonable to assume that the number of DSB per cell nucleus is Poisson-distributed (Virsik and Harder 1981).

For concreteness, consider specifically the number of lethal lesions per cell after an acute dose of low LET radiation. Denote the average number of lethal lesions per cell at a given time by L. Lethal lesion number is said to be Poisson (or Poisson-distributed from cell to cell) if the probability a cell has no lethal lesions is $\exp[-L]$, and, more generally, the probability P_m that a cell has m lethal lesions is

$$P_m = (L^m/m!)e^{-L}, \qquad m = 0, 1, 2, \ldots (0! \equiv 1) \tag{B.1}$$

Note here that m, in contrast to its average L, is an integer.

To illustrate manipulations with the Poisson distribution, here is the way to check from equation (B.1) the claim, made above, that the average over cells of the lethal lesion number m is L. This average is $0 \times P_0 + 1 \times P_1 + 2 \times P_2 + \ldots$. Therefore, with $\langle \ldots \rangle$ denoting averages

$$\langle m \rangle = \sum_{m=0}^{\infty} mP_m = e^{-L} \sum_{m=0}^{\infty} \frac{mL^m}{m!}$$

$$= Le^{-L} \sum_{m=1}^{\infty} \frac{L^{m-1}}{(m-1)!} = Le^{-L}e^{L} = L \tag{B.2}$$

where the Taylor expansion of $\exp[L]$ was used. Comparing the start and finish of equation (B.2) shows the claimed result on averages is correct. Despite the infinite sums involved in manipulations like (B.2) the Poisson distribution is user friendly, having many other useful properties whose proofs are only slightly harder than (B.2). Three basic properties will now be illustrated by examples in lieu of mathematically precise formulations or proofs.

B.2. *The sum of independent Poisson quantities is Poisson*

For example, suppose one kind of lethal lesion is made directly; suppose the number fluctuates a bit

from cell to cell and the lesions are Poisson-distributed with average number L_1; suppose other lethal lesions are made, independently of the first kind, by binary misrepair of DSB, are also Poisson, and have average number L_2; then, adding within each cell, one finds the total lethal lesion number is also Poisson, with average $L_1 + L_2$. Additivity also holds for more summands (Breimann 1969, p. 95). Poisson distributions (and Gaussians) are among the very few distributions which have this useful additivity property.

B.3. *A random thinning of a Poisson quantity is Poisson*

To illustrate what is meant here, suppose that, at $t = 0$, DSB are Poisson-distributed, with some average, say U. Suppose then each DSB undergoes a transition, either being repaired (with probability p) or left unrepaired (with probability $1 - p$), the transitions of different DSB being independent of each other. For this process repaired DSB per cell turn out to be Poisson (with average number Up), and unrepaired DSB are Poisson with average number $U(1 - p)$, both being 'random thinnings' of the original DSB. For the proof, see Breimann (1969, pp. 139–140).

B.4. *The sum of many small, independent Bernoulli quantities is Poisson*

To illustrate what is meant by this formidable-sounding but very useful statement, suppose a cell receives 1 Gy of low LET radiation. Divide the genome into stretches of 1 kbp each. Then in each stretch there is a probability, denote it by p, of no DSB, a very small probability $\approx 1 - p$ of one DSB, and a wholly negligible probability of more than one DSB. The number of DSB in one kbp stretch is effectively a Bernoulli quantity (i.e. can only be zero or one); there are many stretches (about 6×100 of them); the Bernoulli quantity is small ($1 - p$ is something like 0·000005); and the probabilty for one DSB in a given stretch is, at low LET, independent of the behaviour of all the other stretches. The relevant theorem (Breimann 1969, pp. 32–34) says that under these circumstances the sum, i.e. the total number of DSB per cell, is Poisson-distributed. It is this 'sum of many independent improbable quantities' property which, perhaps more than anything else, accounts for the widespread applicability of the Poisson distribution.

B.5. *The Poisson assumptions for the representative model*

In this review, one main application of the Poisson distribution is in Subsection 3.1. There it is assumed that DSB and lethal lesions are Poisson-distributed from cell to cell. These assumptions can be motivated roughly as follows. Consider a single acute dose just before $t = 0$. Then, at low LET, DSB at $t = 0$ will be approximately Poisson (property B.4). Moreover, as the number of DSB in each cell decreases by first order restitution, one has essentially a random thinning so the DSB will remain Poisson as time goes on (property B.3). The lethal lesions made by binary misrepair correspond roughly to having each of many DSB pairs with a very small probability of making a lethal lesion, so the lethal lesions produced should also be Poisson (property B.4). Lethal lesions made by lethal restitution (i.e. by the pathway shown in Figure 2B) are Poisson by property B.4, so the total lethal lesions, as the sum of two independent Poisson quantities, are then also Poisson (property B.2).

This argument that lethal lesions are Poisson distributed only works if, as here, a dicentric and its acentric fragment taken together (Figure 1B) are counted as one lethal lesion, not as two lethal lesions as is done in other treatments (e.g. Albright and Tobias 1985). Poisson statistics are robustly observed for dicentrics at low LET (e.g. Lloyd *et al.* 1987), and are essential to the theory (equation (5)).

Given that DSB are Poisson distributed at all times, it is possible to derive the misrepair reaction rate term κU^2 in equation (2) by an argument which does not rely on $U \gg 1$ (Albright 1989). For U small, the fluctuations of the DSB number from cell to cell can be important, so denote by ν the number of DSB in a particular cell at time t, with per-cell average $\langle \nu \rangle = U(t)$. In each cell, ν is an integer, but the average over cells, $U(t)$, is normally not an integer. For each cell, the number of DSB pairs is the integer $\nu(\nu - 1)/2$. If κ is the per-pair rate of binary misrepair and two DSB are removed in each binary misrepair (Figure 1) the average rate of removing DSB by binary misrepair is $\langle 2\kappa\nu(\nu - 1)/2 \rangle$, i.e.

$$\kappa \langle \nu(\nu - 1) \rangle = \kappa \langle \nu \rangle^2 = \kappa U^2(t) \qquad \text{(B.3)}$$

as assumed in equation (2). In equation (B.3) a Poisson distribution was used to calculate the average, much as in equation (B.2).

The argument given above for Poisson statistics is not exact, and there can be deviations from Poisson distributions for DSB and/or lethal lesions. In typical low LET experiments, the corrections are minor (Harder 1988, Albright 1989, Sachs *et al.* 1992). But to see that they are minor, to handle those low LET situations where they are not minor, and to indicate why many of the arguments of this review fail at high LET, a slightly closer look is worthwhile. There are two main reasons for deviations from Poisson distributions: if the incoming radiation makes a non-

Poisson distribution of DSB; or if damage processing causes DSB and/or lethal lesions to gradually deviate from Poisson behaviour.

B.6. *Microdosimetrically determined deviations from Poisson distributions*

To analyse the first possibility, suppose an acute dose D is delivered just before $t=0$. Each track (i.e. 'event', compare the caption to Figure 2) has certain probabilities of making no DSB, one DSB, two DSB, etc. Attempting to find those probabilities (from the physics of the radiation and the chemistry of the cell, or experimentally), has been the subject of an enormous, long-term effort, which involves microdosimetry heavily (Goodhead 1987, Brenner and Ward 1992, Varma and Chatterjee 1994). Here we need only two facts: (A) gives the probabilities, one can calculate quantities like the average DSB number per track, the average number of DSB pairs per track, etc; (B), at low LET the probability one track makes more than one DSB is often negligible.

Suppose the tracks are Poisson distributed from cell to cell. For identical cell nuclei which present identical cross sections to the radiation, this is essentially just property B.4 one more time. As above, denote by ν the number or DSB in a particular cell at $t=0$, the average of ν being $U(0)$. Using a variance calculation, not too different from the manipulations in equation (B.2), one can prove the following generalization of equation (B.3) (Hug and Kellerer 1966):

$$(1/4)2\kappa\langle\omega(\nu-1)/2\rangle=(\kappa/4)U^2(0)+Y, \qquad \text{(B.4)}$$

where

$$Y=(\kappa/4)\times(\text{average \# of DSB pairs in one track})\times(\text{average \# of tracks/cell})$$

If the probability of making two or more DSB by one track is negligible and the distribution of DSB is Poisson by property B.4, then the average number of DSB pairs per track is negligible, so Y in equation (B.4) is negligible, and equation (B.4) is merely the familiar term $\kappa U^2/4$ in equation (3) for lethal lesion creation rate at $t=0$. In that case (Subsection 4.4) lethal binary misrepairs contribute only to the β term in the LQ approximation, not to the α term. However, if some tracks make more than one DSB, the average number of DSB pairs per track is non-zero, and therefore in equation (B.4) $Y\neq 0$. Then Y contributes extra terms in equations (2) and (3). The contribution is proportional to dose and thus corresponds to α in LQ approximation, since the average number of tracks per cell nucleus is proportional to dose. In short, if a significant fraction of tracks makes more than one DSB, as occurs at high LET but probably not at low LET, the Poisson approximation for DSB per cell does not hold and there is an extra contribution to α in the LQ formalism, i.e. an extra effect directly proportional to dose and independent of dose protraction.

B.7. *Deviations from Poisson distributions caused by damage processing*

Even if an acute dose makes Poisson-distributed DSB, damage processing will cause deviations from Poisson distributions for DSB, and for lethal lesions, at subsequent times. The magnitude of this effect can be estimated by Markov damage processing models which keep track of the details of the statistical distribution of lesions from cell to cell (Albright 1989, Hahnfeldt *et al.* 1992, Sachs *et al.* 1992), and turns out to be quite small in most situations. For example, the Markov analogue of the model in Section 3 can be used to calculate, for the parameters used in Figure 4, the average number of lethal lesions $L(\infty)$, the zero class Z for lethal lesions (i.e. the fraction of cells which at large times have no lethal lesions), and the variance V for cell-to-cell fluctuations of lethal lesions. The result of the calculation for the doses of 0–15 Gy which were used in Figure 4, are the following: L deviates very slightly from the L calculated with the averaged equations (2)–(6) but for practical purposes is identical; for large doses Z is somewhat smaller than $\exp[-L(\infty)]$, as shown in Figure 5, so that, for example, at 15 Gy the Poisson approximation used in the main text (Section 3) overestimates survival by about 20%; and for large doses, V/L, which would be 1·0 for a Poisson distribution gradually becomes less than 1 (Figure 5). These are not large effects, and in a model which has sites (Appendix A.8), the effects of deviations from the Poisson distribution are even smaller (Harder 1988, Hawkins 1996). Overall, for low LET the standard Poisson assumptions are excellent approximations at all times in most relevant situations. Significant deviations usually occur only at doses so high that the kinetic reaction-rate models also have other problems (discussed in Subsection 3.1).

References

ALBRIGHT, N., 1989, A Markov formulation of the repair-misrepair model of cell survival. *Radiation Research*, **118**, 1–20

ALBRIGHT, N. W. and TOBIAS, C. A., 1985, Extension of the time- independent repair-misrepair model of cell survival to high-LET and multicomponent radiation. In *Proceedings of the Berkeley Conference in Honor of Jerzy Neyman and Jack*

Kiefer, Vol. 1, edited by L. M. Le Cam and R. A. Olshen (New York: Wadsworth Inc.), pp. 397–424.

BAUCHINGER, M., 1985, Quantification of low-level radiation exposure by conventional chromosome aberration analysis. *Mutation Research*, **339,** 177–189.

BEDFORD, J. S. and CORNFORTH, M. N., 1987, Relationship between recovery from sublethal X-ray damage and the rejoining of chromosome breaks in normal human fibroblasts. *Radiation Research*, **111,** 406–423.

BOYCE, W. E. and DIPRIMA, R. C., 1997, *Elementary Differential Equations*, 6th ed. (New York: Wiley).

BREIMAN, L., 1969, *Probability and Stochastic Processes* (New York: Houghton Mifflin).

BRENNER, D. J., 1990, Track structure, lesion development, and cell survival. *Radiation Research*, **124,** S29–37.

BRENNER, D. J., HAHNFELDT, P., AMUNDSON, S. A. and SACHS, R. K., 1996, Interpretation of inverse dose-rate effects for mutagenesis by sparsely ionizing radiation. *International Journal of Radiation Biology*, **70,** 447–458.

BRENNER, D. J., HLATKY, L. R., HAHNFELDT, P. J., HALL, E. J. and SACHS, R. K., 1995, A convenient extension of the linear-quadratic model to include redistribution and reoxygenation. *International Journal of Radiation Oncology, Biology and Physics*, **32,** 379–390.

BRENNER, D. J., HLATKY, L. R., HAHNFELDT, P. J., HUANG, Y. and SACHS, R. K., 1997, Dose-response and fractionation/protraction relations: common radiobiological models make similar predictions. *Radiation Research*, submitted for publication.

BRENNER, D. J., HUANG, Y. and HALL, E. J., 1991, Fractionated high dose-rate versus low dose-rate regimens for intracavitary brachytherapy of the cervix: equivalent regimens for combined brachytherapy and external irradiation. *International Journal of Radiation Oncology, Biology and Physics*, **21,** 1415–1423.

BRENNER, D. J. and SACHS, R. K., 1994, Generalized microdosimetric calculations of cell-to-cell variance. *Radiation Protection Dosimetry*, **52,** 21–24.

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.

BRENNER, D. J., WARD, J. and SACHS, R. K., 1994, Track structure, chromosome geometry and chromosome aberrations. In: *Basic Life Sciences,* **63,** *Computational Approaches in Molecular Radiation Biology*. Edited by M. N. Varma and A. Chatterjee (New York: Plenum) pp. 93–113.

BRYANT, P. E., 1995, Rejoining and expression of radiation-induced DNA double strand breaks in mammalian cells. In *Radiation Research 1895–1995*, Vol. 2, edited by U. Hagen, D. Harder, H. Jung and C. Streffer (Wurtzburg: H. Sturtz AG), pp. 368–371.

CHADWICK, K. H. and LEENHOUTS, H. P., 1981, *The Molecular Theory of Radiation Biology*. (Berlin: Springer).

CHADWICK, K. H., MOSCHINI, G. and VARMA, M. N., 1992, *Biophysical Modelling of Radiation Effects* (A. Hilger: Bristol).

CHATTERJEE, A. and HOLLEY, W. R., 1991, Energy deposition mechanisms and biochemical aspects of DNA strand breaks by ionizing radiation, *International Journal of Quantum Chemistry*, **39,** 709–727.

CHEN, A. M., LUCAS, J. N., BRENNER, D. J., HILL, F. S. and SACHS, R. K., 1996, Proximity effects for chromosome aberrations measured by 3-color FISH. *International Journal of Radiation Biology*, **69,** 411–420.

CHEN, A. M., LUCAS, J. N., SIMPSON, P. J., GRIFFIN, C. S., SAVAGE, J. R. K., BRENNER, D. J., HLATKY, L. R. and SACHS, R. K., 1997, Computer simulation of FISH data on chromosome aberrations produced by x-rays or alpha particles. *Radiation Research*, in press.

CORNFORTH, M. N. and BEDFORD, J., 1993, Ionizing radiation damage and its early development in chromosomes. *Advances in Radiation Biology*, Vol. 17, *DNA and Chromatin Damage Caused by Radiation*, edited by J. T. Lett and W. K. Sinclair (San Diego: Academic Press), pp. 423–496.

CURTIS, S. B., 1986, Lethal and potentially lethal lesions induced by radiation—a unified repair model [published erratum appears in *Radiation Research*, **119,** 584, 1989]. *Radiation Research*, **106,** 252–270.

CURTIS, S. B., 1988, The lethal and potentially lethal model—a review and recent development. In *Quantitative Mathematical Models in Radiation Biology*, edited by J. Kiefer (New York: Springer), pp. 146–152.

DALE, R. G., 1985, The application of the linear-quadratic dose-effect equation to fractionated and protracted radiotherapy. *British Journal of Radiology*, **58,** 515–528.

DESCHAVANNE, P. J., FERTIL, B., CHAVAUDRA, N. and MALAISE, E. P., 1990, The relationship between radiosensitivity and repair of potentially lethal damage in human tumor cell lines with implications for radioresponsiveness. *Radiation Research*, **122,** 29–37.

DEWEY, W. C., LING, C. C. and MEYN, R. E., 1995, Radiation-induced apoptosis: relevance to radiotherapy. *International Journal of Radiation Oncology, Biology and Physics*, **33,** 781–796.

DURANTE, M., GEORGE, K., WU, H. and YANG, T. C., 1996, Rejoining and misrejoining of radiation-induced chromatin breaks. I. Experiments with human lymphocytes. *Radiation Research*, **145,** 274–280.

DURANTE, M., GROSSI, G. F., GIALANELLA, G., PUGLIESE, M., NAPPO, M. and YANG, T. C., 1995, Effects of alpha-particles on survival and chromosomal aberrations in human mammary epithelial cells. *Radiation and Environmental Biophysics*, **34,** 195–204.

DUTRILLAUX, B., VIEGAS-PEQUIGNOT, E., PROD´HOMME, M. and SPORTES, M., 1985, Distribution of the various radiation-induced chromosomal rearrangements in relation to the dose and sampling time. *Mutation Research*, **152,** 197–203.

EDWARDS, A. A., MOISEENKO, V. V. and NIKJOO, H., 1996, On the mechanism of the formation of chromosomal aberrations by ionizing radiation. *Radiation and Environmental Biophysics*, **35,** 25–30.

EDWARDS, A. A., VIRSIK-PEUCKERT, P. and BRYANT, P., 1996, Mechanisms of radiation-induced chromosome aberrations. *Mutation Research*, **366,** 117–128.

ERDI, P. and TOTH, J., 1989, *Mathematical Models of Chemical Reactions: Theory and Applications of Deterministic and Stochastic Models*. (Princeton: Princeton University Press).

EVANS, J. W., LIU, X. F., KIRCHGESSNER, C. U. and BROWN, J. M., 1996, Induction and repair of chromosome aberrations in scid cells measured by premature chromosome condensation. *Radiation Research*, **145,** 39–46.

FERTIL, B., REYDELLET, I. and DESCHAVANNE, P. J., 1994, A benchmark of cell survival models using survival curves for human cells after completion of repair of potentially lethal damage. *Radiation Research*, **138,** 61–69.

FORAY, N., BADIE, C., ALSBEIH, G., FERTIL, B. and MALAISE, E. P., 1996, A new model describing the curves for repair of both DNA double-strand breaks and chromosome damage. *Radiation Research*, **146,** 53–60.

FOWLER, J. F., 1989, The linear-quadratic formula and progress in fractionated radiotherapy. *British Journal of Radiology*, **62,** 679–694.

FRANKENBERG-SCHWAGER, M., 1989, Review of repair kinetics for DNA damage induced in eukaryotic cells in vitro by ionizing radiation. *Radiotherapy and Oncology*, **14,** 307–320.

FRIEDL, A. A., KRAXENBERGER, A. and ECKARDT-SCHUPP, F., 1995, An electrophoretic approach to the assessment of the spatial distribution of DNA double-strand breaks in mammalian cells. *Electrophoresis*, **16,** 1865–1874.

GOODHEAD, D. T., 1985, Saturable repair models of radiation action in mammalian cells. *Radiation Research*, **8,** S58–S67.

GOODHEAD, D. T., 1987, Relationship of microdosimetric techniques to applications in biological systems. In *The Dosimetry of Ionizing Radiation*, Vol. II, edited by K. Kase, B. Bjarngard and F. Attix (Orlando: Academic Press), pp. 1–89.

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

GOODWIN, E. H. and CORNFORTH, M. N., 1991, A cytogenetic model of radiation effects. In *Radiation Research, A Twentieth Century Perspective*, Vol. I, edited by J. D. Chapman, W. C. Dewey and G. F. Whitmore (New York: Academic Press), p. 414.

GREINERT, R., DETZLER, E., VOLKMER, B. and HARDER, D., 1995, Kinetics of the formation of chromosome aberrations in X-irradiated human lymphocytes: analyses by premature chromosome condensation with delayed fusion. *Radiation Research*, **144,** 190–197.

GREINERT, R., VOLKMER, B., VIRSIK-PEUCKERT, R. P. and HARDER, D., 1996, Comparative study of the repair kinetics of chromosomal aberrations and DNA strand breaks in proliferating and quiescent CHO cells. *International Journal of Radiation Biology*, **70,** 33–43.

HAGEN, U., 1989, Biochemical aspects of radiation biology, *Experientia*, **45,** 7–12.

HAHNFELDT, P. and HLATKY, L., 1996, Resensitization due to redistribution of cells in phases of the cell cycle during arbitrary radiation protocols. *Radiation Research*, **145,** 134–143.

HAHNFELDT, P., SACHS, R. K. and HLATKY, L. R., 1992, Evolution of DNA damage in irradiated cells. *Journal of Mathematical Biology*, **30,** 493–511.

HALL, E. J., 1994, *Radiobiology for the Radiologist* (Philadelphia: J. B. Lippincott).

HANIN, L. G., PAVLOVA, L. V. and YAKOVLEV, A. Y., 1994, *Biomathematical Problems in Optimization of Cancer Radiotherapy* (Boca Raton: CRC Press).

HARDER, D., 1988, The pairwise lesion interaction model. In *Quantitative Mathematical Models in Radiation Biology*, edited by Jurgen Kiefer (Berlin and New York: Springer-Verlag), pp. 159–170.

HAWKINS, R. B., 1996, A microdosimetric-kinetic model of cell death from exposure to ionizing radiation of any LET, with experimental and clinical applications. *International Journal of Radiation Biology*, **69,** 739–755.

HAYNES, R. H., 1964, Molecular localization of radiation damage relevant to bacterial inactivation. In *Physical Processes in Radiation Biology*, edited by L. Augenstein, R. Mason and B. Rosenberg (New York: Academic Press), pp. 51–72.

HENDRY, J. H. and WEST, C. M., 1995, Implications of delayed reproductive cell death (lethal mutations/genomic instability) for the interpretation of tissue responses. *International Journal of Radiation Biology*, **68,** 363–367.

HLATKY, L., HAHNFELDT, P. and SACHS, R. K., 1994, The influence of time-dependent, stochastic heterogeneity on the radiation response of a cell population. *Mathematical Biosciences*, **122,** 201–220.

HLATKY, L., SACHS, R. and HAHNFELDT, P., 1991, Reaction kinetics for the development of radiation-induced chromosome aberrations. *International Journal of Radiation Biology*, **59,** 1147–1172.

HUG, O. and KELLERER, M., 1966, *Stochastik der Strahlenwirkung* (Berlin: Springer-Verlag).

HUTCHINSON, F., 1995, Analysis of deletions induced in the genome of mammalian cells by ionizing radiation. *Journal of Molecular Biology*, **254,** 372–380.

ILIAKIS, G., 1991, The role of DNA double strand breaks in ionizing radiation-induced killing of eukaryotic cells. *Bioessays*, **13,** 641–648.

KAPPOS, A. and POHLIT, W., 1972, A cybernetic model for radiation reactions in living cells. I. Sparsely-ionizing radiations; stationary cells. *International Journal of Radiation Biology*, **22,** 51–65.

KELLERER, A. M., 1985, Fundamentals of microdosimetry. In *The Dosimetry of Ionizing Radiation*, Vol. I, edited by K. Kase, B. Bjarngard and F. Attix (Orlando: Academic Press), pp. 77–162.

KELLERER, A. M. and ROSSI, H. H., 1972, The theory of dual radiation action. *Current Topics in Radiation Research Quarterly*, **8,** 85–158.

KELLERER, A. M. and ROSSI, H. H., 1978, A generalized formulation of dual radiation action. *Radiation Research*, **75,** 471–488.

KIEFER, J., 1988a, *Quantitative Mathematical Models in Radiation Biology* (Berlin and New York: Springer-Verlag).

KIEFER, J., 1988b, A repair fixation model. In *Quantitative Mathematical Models in Radiation Biology*, edited by J. Kiefer (Berlin and New York: Springer-Verlag), pp. 171–180.

KIEFER, J., 1993, Problems, pitfalls, perspective and potentials of quantitative theoretical models for cellular radiation action. *Mutation Research*, **289,** 27–37.

KIEFER, J. and LOBRICH, M., 1992, The repair fixation model: general aspects and the influence of radiation quality. In *Biophysical Modeling of Radiation Effects*, edited by K. H. Chadwick, G. Moschini and M. N. Varma (New York: Adam Hilger Co.), pp. 211–218.

LEA, D. E., 1946, *Actions of Radiations on Living Cells* (London: Cambridge University Press).

LE BEAU, M. M. and ROWLEY, J. D., 1986, Chromosomal abnormalities in leukemia and lymphoma: Clinical and biological significance. In *Advances in Human Genetics*, edited by H. Harris and K. Hirschhorn (New York: Plenum Press), pp. 1–54.

LLOYD, D. C. and EDWARDS, A. A., 1983, Chromosome aberrations in human lymphocytes: effect of radiation quality, dose and dose rate. In *Radiation-induced Chromosome Damage in Man*, edited by T. Ishihara and M. S. Sasaki (New York: Alan R. Liss), pp. 23–49.

LLOYD, D. C., EDWARDS, A. A., LEONARD, A., DEKNUDT, G. L., VERSCHAEVE, L. *et al.*, 1992, Chromosomal aberrations in human lymphocytes induced *in vitro* by very low doses of X-rays. *International Journal of Radiation Biology*, **61,** 335–343

LLOYD, D. C., EDWARDS, A. A., PROSSER, J. S., BARJAKTAROVIC, N., BROWN, J. K. *et al.*, 1987, A collaborative exercise on cytogenetic dosimetry for simulated whole and partial body accidental irradiation. *Mutation Research*, **179,** 197–208.

LOBRICH, M., RYDBERG, B. and COOPER, P. K., 1995, Repair of X-ray-induced DNA double-strand breaks in specific Not I restriction fragments in human fibroblasts: joining

of correct and incorrect ends. *Proceedings of the National Academy of Science, USA*, **92,** 12 050–12 054.

MEYN, R. E., STEPHENS, L. C. and MILAS, L., 1996, Programmed cell death and radioresistance. *Cancer Metastasis Reviews*, **15,** 119–131.

MICHALIK, V. and FRANKENBERG, D., 1996, Two types of double strand breaks in electron and photon tracks and their relation to exchange-type chromosome aberrations. *Radiation and Environmental Biophysics*, **35,** 163–169.

MOISEENKO, V. V., EDWARDS, A. A. and NIKJOO, H., 1996, Modelling the kinetics of chromosome exchange formation in human cells exposed to ionizing radiation. *Radiation and Environmental Biophysics*, **35,** 31–35.

MORGAN, W. F. and MURNANE, J. P., 1995, A role for genomic instability in cellular radioresistance? *Cancer Metastasis Reviews*, **14,** 49–58.

NELSON, J. M., BRABY, L. A., METTING, N. F. and ROESCH, W. C., 1989, Analyzing the role of biochemical processes in determining response to ionizing radiations. *Health Physics*, **57,** Suppl 1, 369–376.

NEWMAN, H. C., PRISE, K. M., FOLKARD, M. and MICHAEL, B. D., 1997, DNA double-strand break distributions in X-ray and alpha-particle irradiated V79 cells: evidence for non-random breakage. *International Journal of Radiation Biology*, **71**, 347–363.

NORMAN, A. and SASAKI, M. S., 1966, Chromosome-exchange aberrations in human lymphocytes. *International Journal of Radiation Biology*, **11,** 321–328.

NOWELL, P. C., 1997, Genetic alterations in leukemias and lymphomas: impressive progress and continuing complexity. *Cancer Genetics and Cytogenetics*, **94,** 13–19.

OBATUROV, G. M., MOISEENKO, V. V. and FILIMONOV, A. S., 1993, Model of mammalian cell reproductive death. I. Basic assumptions and general equations. *Radiation and Environmental Biophysics*, **32,** 285–294.

OSTASHEVSKY, J. Y., 1993, Cell recovery kinetics for split-dose, multifractionated and continuous irradiation in the DSB model. *International Journal of Radiation Biology*, **63,** 47–58.

PANDITA, T. K. and GEARD, C. R., 1996, Chromosome aberrations in human fibroblasts induced by monoenergetic neutrons. I. Relative biological effectiveness. *Radiation Research*, **145,** 730–739.

PEACOCK, J. H., DE ALMODOVAR, M. R., MCMILLAN, T. J. and STEEL, G. G., 1992, The nature of the initial slope of radiation cell survival curves. *British Journal of Radiology*, **24,** (Suppl), 57–60.

PFEIFFER, P., GOTTLICH, B., REICHENBERGER, S., FELDMANN, E., DAZA, P., DAZA, P., WARD, J. F., MILLIGAN, J. R., MULLENDERS, L. H. and NATARJAN, A. T., 1996, DNA lesions and repair. *Mutation Research*, **366,** 69–80.

PIERCE, D. A., SHIMIZU, Y., PRESTON, D. L., VAETH, M. and MABUCHI, K., 1996, Studies of the mortality of atomic bomb survivors. Report 12, Part I. Cancer: 1950-1990, *Radiation Research*, **146,** 1–27.

PRESTON, R. J., 1990, Mechanisms of induction of specific chromosomal alterations. *Basic Life Sciences*, **53,** 329–336.

RABBITTS, T. H., 1994, Chromosomal translocations in human cancer. *Nature*, **372,** 143–149.

RADIVOYEVITCH, T., 1997, Mathematical models of misrejoining DNA double-strand breaks and DNA fragment size distributions. Ph.D. Thesis, Medical University of South Carolina, South Carolina, USA, pp. 15–34.

RADIVOYEVITCH, T., HOEL, D. G., HAHNFELDT, P. J., RYDBERG, B., CHEN, A. M. and SACHS, R. K., 1997, Double-strand break misrejoining after x-irradiation: relating moderate to very high doses by a Markov model, *Radiation Research*, submitted for publication.

REDDY, N. M. S., MAYER, P. J. and LANGE, C. S., 1990, The saturated repair kinetics of Chinese hamster V79 cells suggests a damage accumulation-interaction model of cell killing. *Radiation Research*, **121,** 304–311.

RIPLEY, B. D., 1987, *Stochastic Simulation* (New York: John Wiley & Sons, Inc.).

ROSSI, H. H. and ZAIDER, M., 1988, Saturation in dual radiation action. In *Quantitative Mathematical Models in Radiation Biology*, edited by J. Kiefer (New York: Springer), pp. 111–118.

ROSSI, H. H. and ZAIDER, M., 1996, *Microdosimetry and its Applications* (New York: Springer).

SACHS, R. K. and BRENNER, D. J., 1993, Effect of LET on chromosomal aberration yields. I. Do long-lived, exchange-prone double strand breaks play a role? *International Journal of Radiation Biology*, **64,** 677–688.

SACHS, R. K., CHEN, A. M. and BRENNER, D. J., 1997, Review: proximity effects in the production of chromosome aberrations by ionizing radiation. *International Journal of Radiation Biology*, **71,** 1–19.

SACHS, R. K., CHEN, P.-L., HAHNFELDT, P. J. and HLATKY, L. R., 1992, DNA damage caused by ionizing radiation. *Mathematical Biosciences*, **112,** 271–303.

SANCHEZ–REYES, A., 1992, A simple model of radiation action in cells based on a repair saturation mechanism. *Radiation Research*, **130,** 139–147.

SAVAGE, J. R., 1995, The transmission of FISH-painted patterns derived from complex chromosome exchanges. *Mutation Research*, **347,** 87–95.

SAVAGE, J. R. K., 1996, Insight into sites. *Mutation Research*, **366,** 81–95.

SHNEIDER, D. O. and WHITMORE, G. F., 1963, Comparative effects of neutrons and X-rays on mammalian cells. *Radiation Research*, **18,** 286–306.

SCHWARTZ, J. L., 1992, The radiosensitivity of the chromosomes of the cells of human squamous cell carcinoma cell lines. *Radiation Research*, **129,** 96–101.

SHADLEY, J. D., AFZAL, V. and WOLFF, S., 1987, Characterization of the adaptive response to ionizing radiation induced by low doses of X rays to human lymphocytes. *Radiation Research*, **111,** 511–517.

SHELLABARGER, C. J., STONE, J. P. and HOLTZMAN, S., 1986, Experimental carcinogenesis in the breast. In *Radiation Carcinogenesis*, edited by A. C. Upton, R. E. Albert, F. B. Burns and R. E. Shore (New York: Elsevier), pp. 169–180.

SIMPSON, P. J. and SAVAGE, J. R. K., 1996, Dose-response curves for simple and complex chromosome aberrations induced by X-rays and detected using fluorescence *in situ* hybridization. *International Journal of Radiation Biology*, **69,** 429–436.

SONTAG, W., 1990, Comparison of six different models describing survival of mammalian cells after irradiation. *Radiation and Environmental Biophysics*, **29,** 185–201.

STEEL, G. G., 1991, The ESTRO Breur lecture. Cellular sensitivity to low dose-rate irradiation focuses the problem of tumour radioresistance. *Radiotherapy and Oncology*, **20,** 71–83.

STEEL, G. G., 1996, From targets to genes: a brief history of radiosensitivity. *Physics in Medicine and Biology*, **41,** 205–222.

THAMES, H. D., 1985, An 'incomplete-repair' model for survival after fractionated and continuous irradiations. *International Journal of Radiation Biology*, **47,** 319–339.

THAMES, H. D. and HENDRY, J. H., 1987, *Fractionation in Radiotherapy* (London and New York: Taylor & Francis).

TOBIAS, C. A., 1985, The repair-misrepair model in radiobiology: comparison to other models. *Radiation Research*, **8,** S77–S95.

TOBIAS, C. A., BLAKELY, E. A., NGO, F. Q. H. and YANG, T. C. Y., 1980, The repair-misrepair model of cell survival. In *Radiation Biology in Cancer Research*, edited by R. E. Meyn and H. R. Withers (New York: Raven Press), pp. 195–229.

TUCKER, S. L. and TRAVIS, E. L., 1990, Comments on a time-dependent version of the linear-quadratic model [see comments]. *Radiotherapy and Oncology*, **18,** 155–163.

UPTON, A. C., RANDOLPH, M. L., CONKLIN, J. W., KASTENBAUM, M. A., SLATER, M. *et al.*, 1970, Late effects of fast neutrons and gamma-rays in mice as influenced by the dose rate of irradiation: induction of neoplasia. *Radiation Research*, **41,** 467–491.

VAN RONGEN, E., TRAVIS, E. L. and THAMES, H. D. JR., 1995, Repair rate in mouse lung after clinically relevant radiation doses per fraction. *Radiation Research*, **141,** 74–78.

VARMA, M. N. and CHATTERJEE, A., 1994, *Computational Approaches in Molecular Radiation Biology: Monte Carlo Methods* (New York: Plenum Press).

VIRSIK, R. P. and HARDER, D., 1981, Statistical interpretation of the overdispersed distribution of radiation-induced dicentric chromosome aberrations at high LET. *Radiation Research*, **85,** 13–23.

WARD, J. F., 1990, The yield of DNA double strand breaks produced intracellularly by ionizing radiation: a review. *International Journal of Radiation Biology*, **57,** 1141–1150.

WARD, J. F., 1994, Interplay of damage complexity and damage repair determine RBE. In *Proceedings of the NIRS International Seminar on the Application of Heavy Ion Accelerators to the Radiation Therapy of Cancer*, edited by T. Kanai and E. Takada, NIRS-M-103 (National Institute of Radiological Sciences: Chiba-shi 263, Japan) pp. 181–184.

WEST, C. M., 1995, Invited review: intrinsic radiosensitivity as a predictor of patient response to radiotherapy. *British Journal of Radiology*, **68,** 827–837.

WU, H., DURANTE, M., GEORGE, K., GOODWIN, E. and YANG, T., 1996, Rejoining and misrejoining of radiation-induced chromatin breaks. II. Biophysical model. *Radiation Research*, **145**, 274–280.

WITHERS, H. R., 1992, Biologic basis for radiation therapy. In *Principles and Practise of Radiation Oncology*, edited by C. A. Perez and L. W. Brady (Philadelphia: Lippincott), pp. 64–96.

YAKOVLEV, A. Y. and ZORIN, V., 1988, *Computer Simulation in Cell Radiobiology* (Berlin: Springer-Verlag).

ZACKRISSON, B., 1992, Radiobiological cell survival models. A methodological overview. *Acta Oncologica*, **31,** 433–441.

ZAIDER, M., WUU, C. S. and MINERBO, G. N., 1996, The combined effects of sublethal damage repair, cellular repopulation and redistribution in the mitotic cycle. I. Survival probabilities after exposure to radiation. *Radiation Research*, **145**, 457–466.