

# Interpretation of inverse dose-rate effects for mutagenesis by sparsely ionizing radiation

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**Abstract.** An inverse dose-rate effect has sometimes been observed for mutagenesis in cells exposed to  $\gamma$ -rays. We model such data quantitatively with the key assumption that the effect is caused in cycling cells by correlated variations in sensitivity across the cell cycle, for both mutation and killing. We quantify this approach using the LQR (linear-quadratic + resensitization) formalism, which describes the response to radiation of a heterogeneous cell population. This model is applied to an exponentially growing population. We compare its predictions with dose- and dose-rate dependent mutation data and show that it can well fit the observed inverse dose-rate effect, as well as providing an explanation of why inverse dose-rate effects have been seen in some experiments, but not in others. The actual values of the model parameters emerging from the analysis are reasonable in magnitude, based on their biological interpretations. We conclude that the LQR model can quantify cell-cycle redistribution effects without over-parameterization, and that the data favour a correlation explanation of inverse dose-rate effects for mutagenesis by low-LET radiation. It is less clear that this explanation is appropriate to high-LET radiation-induced oncogenic transformation, although all potential explanations of inverse dose-rate effects predict that, at appropriately low doses, no dose-rate effects of any kind are expected.

## 1. Introduction

Recently, Amundson and Chen (1996) reported dose-rate effects for mutagenesis by low-LET ionizing radiation. Their data on mutation induction at the *hprt* locus in the WTK1 human lymphoblast cell line give a clear-cut example of an inverse dose-rate effect, where a decrease in dose-rate can produce an increase in effect. Similar results for  $\gamma$ -ray-induced mutagenesis in rodent cells have previously been observed by Lorenz *et al.* (1993), Crompton *et al.* (1990), and Furono-Fukushi *et al.*

(1988); however, as we discuss below, 'sparing' dose-rate effects have also been reported where the mutation rate decreases monotonically with decreasing dose-rate.

Following Rossi and Kellerer (1986), inverse dose-rate effects have often been explained on the basis of a 'window of sensitivity' in the cell cycle (Brenner and Hall 1990). During a protracted exposure, extra cells can enter such a sensitive window by cell cycle progression, resulting in the effects of irradiation being enhanced as the irradiation time is increased. Such enhancement requires some kind of 'saturation', meaning here that extra hits to a cell result in a less than a proportionate increase in the probability of a damage endpoint. Such saturation can produce an inverse dose-rate effect through a larger number of wasted hits during acute exposure to cells in a sensitive window, compared with a protracted exposure.

For low-probability endpoints such as mutation or oncogenic transformation, this explanation of inverse dose-rate effects requires the existence of short 'windows' of ultrasensitivity in the cell cycle (Rossi and Kellerer [1986] speculated on a 'window' of  $\sim 10$  min, in which cells were more than an order of magnitude more sensitive than in the rest of the cycle), and/or the existence of some intermediate, saturable damaged state. There are some indications that such intermediate states do exist in the complex process of radiation oncogenesis (Kennedy 1985, Kamiya *et al.* 1995, Selvanayagam *et al.* 1995), although their nature has yet to be elucidated. For mutagenesis, however, there are unlikely to be such intermediate saturable states, nor is there evidence for extremely short windows within the cell cycle of ultrasensitivity to mutagenesis.

In this paper, therefore, we consider a somewhat different, though conceptually related, model for the inverse dose-rate effect in radiation-induced mutagenesis at particular loci, in which the required saturation is indirect, occurring for cell killing but observed through mutation frequencies. The key

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assumption that we make is that cell killing and mutagenesis are positively correlated in their variations through the cell cycle (Oftedal 1968). There is direct evidence (Figure 1a) that sensitivity variations through the cycle for cell killing are closely mirrored by corresponding variation for mutagenesis (Chuang and Liber 1995, Jostes *et al.* 1980, Watanabe and Horikawa 1980, Burki 1980). In the most detailed of these studies—in the sense of covering the most time points—the data of Jostes *et al.* (1980) exhibit an estimated sample correlation coefficient between mutation induction and  $-\log(\text{cell survival})$  of 0.84. For the endpoints that we shall analyse in this paper (survival and mutation at the *hprt* locus in WTK1 human

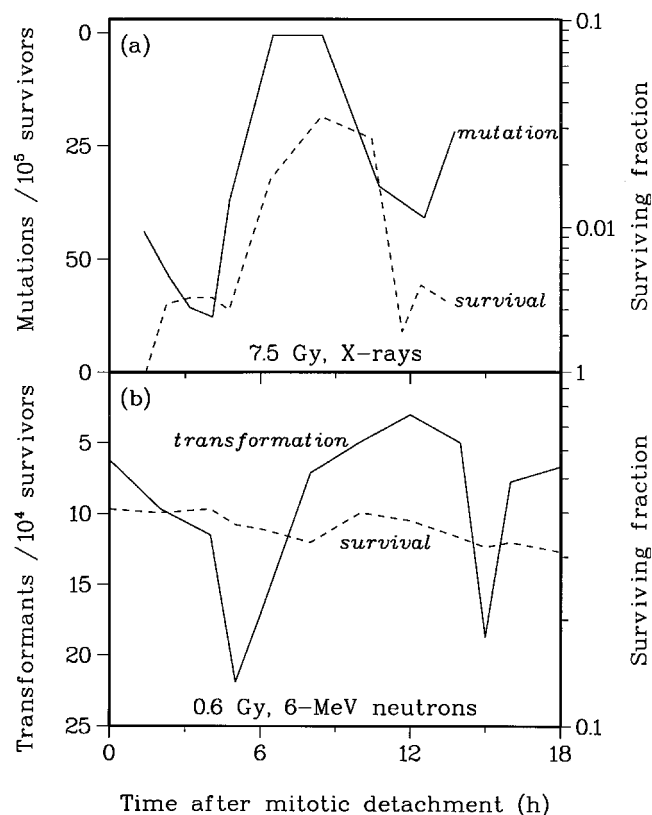


Figure 1. (a) X-ray-induced surviving fraction, and yield of 6-thioguanine-resistant mutant clones per survivor, as a function of cell-cycle position (time after mitotic detachment) in CHO cells (from Burki 1980). A correlated variation in radiosensitivity (estimated sample correlation coefficient = 0.68) is apparent. Similar correlations are reported by Burki at a lower dose, as well as by Chuang and Liber (1995), Jostes *et al.* (1980), and Watanabe and Horikawa (1980). (b) Neutron-induced surviving fraction and yield of oncogenically transformed cells per survivor as a function of cell-cycle position in  $C_3H10T_1/2$  cells (from Miller *et al.* 1995). No correlated variation in radiosensitivity is apparent (estimated sample correlation coefficient = 0.11).

lymphoblast cells) the corresponding estimated correlation coefficient was 0.61 (Chuang and Liber 1995).

A positive correlation through the cell cycle between killing and mutagenesis implies that, for an acute irradiation, cells which have undergone mutation are preferentially killed, and cells resistant to killing have a below average chance of mutagenesis. So for acute exposure, the correlation between killing and mutagenesis decreases the observed mutation rate per surviving cell. By contrast, at low dose-rates, cell cycling can cause mutated cells to progress to resistant phases before they are killed, resulting in previously resistant surviving cells progressing to a sensitive part of the cycle, where they can undergo mutagenesis. Overall, as we shall quantify, decreasing the dose-rate in such situations increases the mutation rate per surviving cell, i.e. the correlations cause an inverse dose-rate effect. A simple hypothetical numerical example is given in the next section.

The model of these processes that we have outlined and will quantify is not unlike that suggested by Elkind (1994); in analysing the observed inverse dose-rate effect for neutron-induced oncogenic transformation, Elkind proposed the existence of a sensitive period in the cell cycle for both oncogenesis and cell killing. Available high-LET radiation data, however (Miller *et al.* 1995, Redpath *et al.* 1995, Pazzaglia *et al.* 1996), do not give any indication of a significant correlation across the cell cycle between cell killing and oncogenic transformation, and indeed show only minor variations in killing across the cell cycle (Figure 1b). Consequently the current approach may well be more applicable to low-LET radiation-induced mutagenesis, for which there is evidence of a correlation with cell killing throughout the cell cycle.

## 2. Materials and methods

### 2.1. Numerical example

To show more concretely that a correlation mechanism can produce inverse dose-rate effects, we consider (Table 1) an idealized 'split-dose' experiment in a hypothetical cell population having two cycle states, one of which is highly sensitive to cell killing and mutagenesis, and one which is not. We suppose that the two states, 1 and 2, have equal time duration, and that the mitotic processes are such that the two states are equally populated prior to irradiation (Steel 1977). We will further suppose that a dose,  $d$ , to cells in state 1

Table 1. Hypothetical example showing how an inverse dose–rate effect can be caused by correlated changes through the cell cycle in both number of surviving cells ( $s$ ), and yield of mutants ( $m$ ). Half the initial cycling cell population are in a sensitive phase in which a dose  $d$  causes a survival of 0.5 and a mutation frequency at a particular locus, per survivor, of  $10^{-4}$ , whilst the other half are in a resistant phase in which this dose causes no killing or mutagenesis

Dose	$10^7$ cells initially in sensitive phase <sup>a</sup>	$10^7$ cells initially in non-sensitive phase <sup>b</sup>	Overall population (initially $2 \times 10^7$ )
$d$ (acute)	$s = 0.5 \times 10^7$ $m = (0.5 \times 10^7) (10^{-4})$ $= 500$	$s = 10^7$ $m = 0$	$s = 1.5 \times 10^7$ $m = 500$ $m/s = M = 3.3 \times 10^{-5}$
$2d$ (acute) <sup>c</sup>	$s = 0.25 \times 10^7$ $m = (0.25 \times 10^7) (2 \times 10^{-4})$ $= 500$	$s = 10^7$ $m = 0$	$s = 1.25 \times 10^7$ $m = 500$ $m/s = M = 4 \times 10^{-5}$
$d + \text{delay}^d + d$	$s = 0.5 \times 10^7$ $m = (0.5 \times 10^7) (10^{-4})$ $= 500$	$s = 0.5 \times 10^7$ $m = (0.5 \times 10^7) (10^{-4})$ $= 500$	$s = 10^7$ $m = 1000$ $m/s = M = 10^{-4}$

<sup>a</sup> Cells in sensitive phase, exposed to a dose  $d$ , have a surviving fraction of 0.5 and a mutation yield at a particular locus per surviving cell of  $10^{-4}$ .

<sup>b</sup> Cells in non-sensitive phase, exposed to a dose  $d$ , have a surviving fraction of 1.0 and zero mutation yield.

<sup>c</sup> For simplicity, we have assumed linear relations between dose and mutation frequency per survivor, and between dose and the logarithm of survival. These assumptions are not necessary for the argument.

<sup>d</sup> Delay sufficient for all cells in sensitive phase to move to non-sensitive phase, and vice versa.

kills half the cells and causes mutagenesis at a particular locus in 1 in 10,000 surviving cells (i.e. a mutation frequency of  $10^{-4}$ ), while that same dose to cells in state 2 produces only negligible killing and mutagenesis. These assumptions exemplify a positive correlation between killing and mutagenesis, the state that is sensitive to killing being the one that is sensitive to mutation.

In this idealized system, let us now consider the results of a split-dose experiment in which the results of an exposure to dose  $2d$  are compared with the results of an exposure to a dose  $d$ , followed by a delay, followed by an exposure to another dose  $d$ .

After an acute dose of  $2d$  (i.e.  $d$  immediately followed by the second dose  $d$ ), 0.25 of the cells ( $0.5 \times 0.5$ ) in state 1 survive, and of these about  $2 \times 10^{-4}$  will have undergone mutagenesis at the locus under study (see footnote c of Table 1). None of the cells in state 2 will be killed or mutated. It is simple (Table 1) to estimate the mutation frequency,  $M$ , per surviving cell for the population as a whole, which is  $4 \times 10^{-5}$ .

For the corresponding split-dose experiment, suppose we deliver the first dose  $d$ , then wait about half a cell cycle, until cycling has interchanged all the cells in the two compartments, and then deliver the second dose  $d$ . Now the overall mutation frequency per surviving cell, computed as above (Table 1), is  $10^{-4}$ , more than twice as large as for the acute dose  $2d$ . This greater mutation frequency per surviving cell for a protracted exposure corresponds to an inverse

dose-rate effect, caused by the correlation between killing and mutagenesis.

## 2.2. Quantifying the effects of cellular progression

In a more realistic situation, in order to quantify dose-rate effects caused by redistribution in a mutagenesis/killing correlation model, one would in principle need a great deal of detailed information. Specifically, complete details of killing and mutagenesis sensitivities throughout the cell cycle would be needed, as well as information on the average and distribution of cell-cycle times. Not only do we not have such detailed information, but also such an approach would be so over-parameterized as to be of little practical use.

However, a tractable model for the effects of cell-cycle redistribution during a protracted exposure has been introduced by Hlatky *et al.* (1994), and recently applied by Brenner *et al.* (1995). The model, which uses a minimum of adjustable parameters, is based on the fact that in an initially exponentially cycling cell population which is perturbed, redistribution generally tends to restore the original sensitivity. For example, suppose in a log-phase population that all the cells in a sensitive part of the cell cycle are inactivated by a single acute dose. Immediately after irradiation, the radiosensitivity of the surviving population will be decreased but, as the population loses synchrony, the log-phase age distribution will be gradually restored (Steel 1977), and the average sensitivity

of the population eventually increases back to its pre-perturbation value. This process is termed resensitization (Hlatky *et al.* 1994).

The resensitization model based on this observation generalizes the usual 'linear-quadratic plus time' (LQ) model (e.g. Thames and Hendry 1987, Travis and Tucker 1987) to include the effects of redistribution (and, in other applications, the effects of tumour reoxygenation); the model is therefore abbreviated LQR (Brenner *et al.* 1995), for 'LQ + Resensitization'. In addition to the usual LQ parameters, there are two extra resensitization parameters, which are (1) an overall amplitude corresponding, roughly, to the variance of radiosensitivity throughout the cell cycle, and (2) an over-all resensitization time, typically of the same order of magnitude as the cell-cycle time.

As we discuss below, by assuming that cell killing and mutagenesis are positively correlated, the LQR survival model can be adapted to analyse mutation frequencies (Hahnfeldt and Sachs 1996). We will here apply the resulting LQR correlation model to the mutagenesis data of Amundson and Chen (1996) on the WTK1 human cell line. It will be seen that the data strongly support the idea of a correlation between killing and mutagenesis, that the LQR correlation model can correctly predict the main features of the mutagenesis data, and that the parameters obtained by fitting these data are biologically reasonable.

### 2.3. The LQR model for cell killing

As discussed, we use the LQR model, which allows us to take into account effects due to redistribution through the cell cycle. The conceptual basis for the LQR model is discussed above, and its mathematics are reviewed in the Appendix. The fundamental equation for survival, given in equation 1, depends on the following parameters:

- (A) Coefficients  $\alpha_0$  and  $\beta$  of the usual LQ model. (Here, following Schultheiss *et al.* [1987], we use  $\alpha_0$ , rather than  $\alpha$ , to denote an average value for a radiobiologically diverse population.)
- (B) A characteristic time,  $T_R$ , for sublethal damage repair.
- (C) An exponential growth rate,  $\lambda$ , for the cell population.
- (D) A resensitization amplitude  $\frac{1}{2}\sigma^2$  (with units  $\text{Gy}^{-2}$ ), where  $\sigma^2$  is a variance in  $\alpha_0$  due, for example, to variations in radiosensitivity in phases of the different cell cycle. In cases we

have examined,  $\sigma^2$  tends to be smaller than  $\beta$ , but both have the same order of magnitude (Brenner *et al.* 1995).

- (E) A characteristic resensitization time  $T_S$ , essentially corresponding to the time needed for a perturbation of the cell population to die away as the cells cycle and synchrony is lost, gradually restoring the radiosensitivity pattern and average radiosensitivity of the unperturbed population. In cases we have examined  $T_S$  tends to be in the range 1–10 h, with one case >50 h (Brenner *et al.* 1995).

The LQR model can be applied to any combination of acute fractions and time-dependent irradiation. Specifically (see Appendix), the survival,  $S$ , at time  $T$  can be written

$$\ln(S) = -\alpha_0 D - \beta G_R D^2 + \frac{1}{2}\sigma^2 G_S D^2 + \lambda T, \quad (1)$$

where the function  $G_x$  ( $x = R$  or  $S$ , denoting, respectively, repair/misrepair or resensitization) is the generalized Lea-Catcheside function, which can be defined for any radiation protocol (Brenner *et al.* 1991); for the case of a continuous uniform exposure over a time  $T$ , it is (Lea and Catcheside 1942)

$$G_x = 2(T/T_x)^2 [(T/T_x) - 1 + e^{-(T/T_x)}]. \quad (2)$$

Thus equation 1, used with equation 2, gives an expression for the survival of a radiobiologically diverse time-dependent cell population, after protracted uniform irradiation. In equation 1,  $\alpha_0$  describes one-track action,  $\beta$  describes damage subject to sublethal damage repair/misrepair (two-track action),  $\sigma^2$  describes cell-cycle redistribution (or, more generally, resensitization), and  $\lambda$  describes cellular proliferation.

It should be noted that the form of the resensitization ( $\sigma^2$ ) term in equation 1 is the same, though with a crucial difference in sign, as the sublethal damage repair/misrepair ( $\beta$ ) term. It can be seen from the difference in signs of these two terms in equation 1 that repair and resensitization have opposite effects—repair leads to less damage as irradiation is prolonged ('sparing' dose-rate effect), but redistribution leads to more damage (inverse dose-rate effect).

### 2.4. Extension of the LQR approach for mutagenesis

Our basic assumption is that the rate of mutation at any given locus, per surviving cell, is proportional to the rate of cell killing, with the same proportionality constant during any phase of the cell cycle. Whilst this is clearly an idealization,

available data suggest that it is not unreasonable (Burki 1980, Jostes *et al.* 1980, Watanabe and Horikawa 1980, Chuang and Liber 1995) (Figure 1a). Mathematically, we assume that whenever a radiation exposure causes a fraction,  $f$ , of cells to be removed from the clonogenic, non-mutated cell population, this fraction is subdivided between the (much smaller) fraction  $\varepsilon f$  that underwent mutagenesis, and the remaining fraction,  $(1 - \varepsilon)f$ , that became non-clonogenic. Here the constant,  $\varepsilon$ , is very small in value (as we discuss below, our data analysis suggests that  $\varepsilon \sim 4 \times 10^{-5}$ ), so the change in the non-mutant cell population due to mutagenesis is negligible compared with the change due to killing; however, the strict proportionality assumed between killing and mutagenesis for all phases of the cell cycle has important consequences for the predicted mutation rate.

To avoid extra adjustable parameters, we will also assume that mutated cells have the same intrinsic cell-cycle kinetics and radiosensitivity as the non-mutant population. Under these assumptions the fraction,  $M$ , of surviving cells which are mutated is (see Appendix)

$$M = \varepsilon[\alpha_0 D + \beta G_R D^2 - \sigma^2 G_S D^2], \quad (3)$$

where  $G_x$  ( $x = R$  or  $S$ , denoting, respectively, repair/misrepair or re-sensitization) is the same generalized Lea-Catcheside function used in equation 1, and defined, for continuous uniform irradiation, in equation 2.

Our remarks describing the different terms in equation 1 for cell killing, also apply to equation 3, describing mutagenesis. As before, sublethal damage repair/misrepair, described by the term  $\beta G_R D^2$ , leads to a sparing dose-rate effect. The re-sensitization term,  $\sigma^2 G_S D^2$  in equation 3, corresponds to an inverse dose-rate effect.

Equation 3 is the expression that we shall fit to the dose-rate data; given that it describes a ratio, and involves both mutagenesis and survival, equation 3 is surprisingly similar to the expression for log-survival (equation 1). The differences are (1) an overall factor  $-\varepsilon$ , which is the proportionality constant relating log-survival to mutation rate at low doses, (2) the absence of a proliferation term (due to the fact that equation 3 describes a ratio between the mutated and the surviving populations which are assumed to be growing at equal rates), and (3) a missing factor  $\frac{1}{2}$  in the last ( $\sigma^2$ ) term.

It is interesting to note that for large times,  $T$ , specifically if  $T \gg T_R$  and  $T \gg T_S$ , equation 3 simplifies to

$$M = \varepsilon[\alpha_0 D + (2/T)(\beta T_R - \sigma^2 T_S)D^2]. \quad (4)$$

For the endpoint of mutation yield per survivor, this expression shows that there would be, respectively, a sparing or an inverse dose-rate effect at long exposure times, depending on whether  $\beta T_R$  is less than or greater than  $\sigma^2 T_S$ . It can easily happen that both terms are comparable in size (typically  $\beta > \sigma^2$  but  $T_R < T_S$ ), so either situation is quite possible. Such considerations may underlie the fact (Lorenz *et al.* 1993) that in studies of the dose-rate dependence of mutagenesis, inverse dose rate effects have been reported (Furono-Fukushi *et al.* 1988, Crompton *et al.* 1990, Amundson and Chen 1996), as well as 'conventional' sparing dose-rate effects (e.g. Nakamura and Okada 1981, Thacker and Stretch 1983, Evans *et al.* 1990, Lorenz *et al.* 1993), and even an absence of any dose-rate effect (Evans *et al.* 1985, König and Kiefer 1988).

### 3. Results

#### 3.1. Comparison of model with experimental results

The model predictions, given by equation 3 for mutation yield, and equation 1, for cellular survival, will now be compared with measured mutagenesis and survival data (Amundson and Chen 1996) in the WTK1 human cell line. It may be noted that a smaller inverse dose-rate effect for mutagenesis was also observed by Amundson and Chen (1996) for a second human lymphoblastoid cell line, TK6.

The entire WTK1 mutagenesis data set, for all dose-rates and doses, was globally fitted to equation 3 using a standard simulated-annealing technique (Kirkpatrick *et al.* 1983). As can be seen from equation 3, the fit has give parameters ( $\varepsilon\alpha_0$ ,  $\varepsilon\beta$ ,  $\varepsilon\sigma^2$ ,  $T_R$ ,  $T_S$ ). The data, model fit and parameter values are given in Figure 2. It can be seen that the essential features of the data are well reproduced, specifically the sparing effect produced by protracting the acute exposure, and the inverse dose-rate effect at lower dose rates. The parameter values themselves are also quite reasonable, with an  $\alpha_0/\beta = 1.6$  Gy, a characteristic value for sublethal damage repair of  $T_R \approx 9$  min, and a characteristic time for redistribution of  $T_S \approx 5.4$  h.

The basis of the model described here is that the same parameter set [ $\alpha_0$ ,  $\beta$ ,  $\sigma^2$ ,  $T_R$ ,  $T_S$ ] applies both to mutagenesis and to survival. So, having obtained the parameter set [ $\varepsilon\alpha_0$ ,  $\varepsilon\beta$ ,  $\varepsilon\sigma^2$ ,  $T_R$ ,  $T_S$ ] from the mutation data (Figure 2), we apply these same parameter values to the corresponding dose-rate dependent survival data of Amundson and Chen

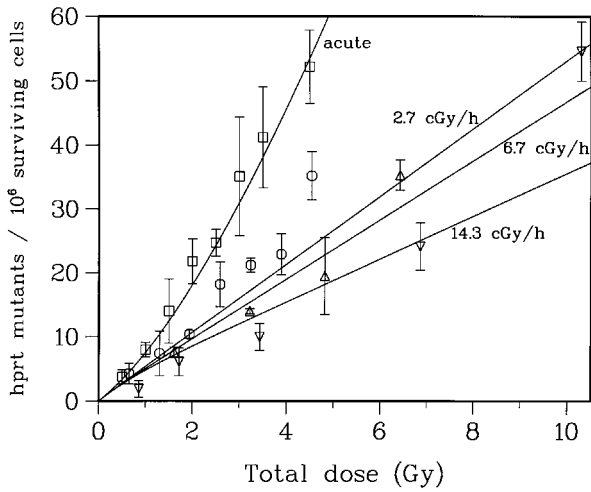


Figure 2. Measured yield per surviving cell of mutants at the *hprt* locus in WTK1 human cells as a function of  $\gamma$ -ray dose and dose-rate (from Amundson and Chen 1996).  $\square$ , Acute exposure, 99 cGy/min;  $\nabla$ , 14.3 cGy/h;  $\triangle$ , 6.7 cGy/h;  $\circ$ , 2.7 cGy/h. The curves represent a global fit to the experimental data using equations 2 and 3. Parameters derived from the fit are  $\varepsilon\alpha_0 = 5.8 \times 10^{-6} \text{ Gy}^{-1}$ ,  $\varepsilon\beta = 3.5 \times 10^{-6} \text{ Gy}^{-2}$ ,  $\varepsilon\sigma^2 = 1.6 \times 10^{-6} \text{ Gy}^{-2}$ ,  $T_R = 0.14 \text{ h}$ ,  $T_S = 5.4 \text{ h}$ .

(1996). Thus we fit the data set shown in Figure 3 to equation 1, using the parameter values obtained from the mutation-data fit [ $\varepsilon\alpha_0$ ,  $\varepsilon\beta$ ,  $\varepsilon\sigma^2$ ,  $T_R$ ,  $T_S$ ], and thus a single remaining free parameter,  $\varepsilon$ . The results, shown in Figure 3, show an excellent

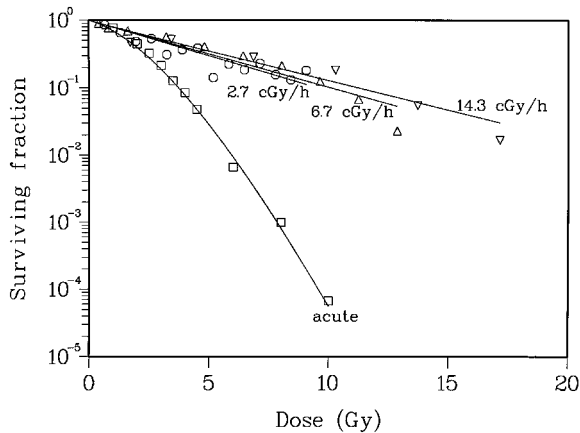


Figure 3. Measured surviving fractions for WTK1 cells exposed to  $\gamma$ -rays as a function of dose and dose-rate (from Amundson and Chen 1996). Symbols are as in Figure 2. The curves represent a one adjustable parameter ( $\varepsilon$ ) global fit of equations 1 and 2 to the data using the other LQR parameters fixed to be those derived in the fit of equations 2 and 3 to the mutation data (see Figure 2). The estimated  $\varepsilon$  parameter (low-dose slope relating log-survival to mutation frequency) is  $4.3 \times 10^{-5}$ .

single-parameter fit to the data. The single parameter,  $\varepsilon$ , whose estimated value is  $4 \times 10^{-5}$ , is the low dose (high survival) slope relating log-survival to mutation rate, as discussed in the next section.

### 3.2. Correlations between killing and mutagenesis

In equation 1 for log-survival, we shall henceforth omit the proliferation term,  $\lambda T$ , reflecting the way the experimental data were processed (Amundson and Chen 1996). Comparing equation 1 for survival with equation 3 for mutation induction, it is clear that there is a strong similarity in the theoretical expressions for the two endpoints. Apart from the overall factor  $-\varepsilon$ , the one-track terms (involving  $\alpha_0$ ) are identical, as are the dose-rate dependent repair terms involving  $\beta$ . The resensitization terms have the same dose-rate dependence  $G$ , and amplitude  $\sigma^2$ , the difference being a factor of  $\frac{1}{2}$ .

Comparing equations 1 and 3, then, this model predicts that a combined scatterplot of the data, for all dose rates, of log-survival against 'modified' mutation frequency,  $M'$ , should fall on the same straight line, independent of dose-rate, with slope  $-\varepsilon$ , i.e.

$$M' = -\varepsilon \ln(S), \quad (5)$$

where the 'modified' mutation rates,  $M'$ , are the actual mutation rates scaled by the factor

$$\frac{\alpha_0 D + \beta G(T_R)D^2 - \frac{1}{2}\sigma^2 G(T_S)D^2}{\alpha_0 D + \beta G(T_R)D^2 - \sigma^2 G(T_S)D^2}. \quad (6)$$

Such a scatter plot is shown in Figure 4a, and log-survival does indeed show a strong correlation, independent of dose-rate, with 'modified' mutation (estimated sample correlation coefficient = 0.90).

In fact, the scaling factor given in equation 6 is generally close to unity, except at very large doses, and so Figure 4b shows an 'uncorrected' scatter plot of log-survival against observed mutation rate, again for all different dose-rates together. Such a plot was displayed in the original paper by Amundson and Chen (1996). It is apparent from Figure 4b that the 'uncorrected' scatter plot (in which the estimated sample correlation coefficient is 0.88) is very similar to the 'corrected' one (Figure 4a), suggesting that the simpler scatter plot of log-survival against mutation rate, for all dose-rates together, is a useful and potentially informative way to view such dose-rate data.

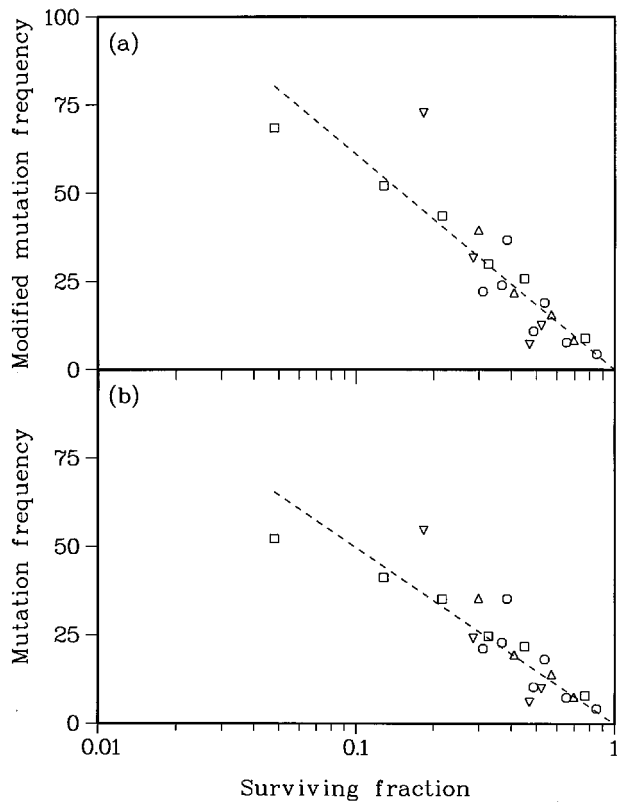


Figure 4. Scatter plots of mutation yield at the *hprt* locus per  $10^6$  surviving cells (data from Figure 2) as a function of cell survival for all dose rates examined. Symbols are as in Figure 2. In (a), each of the measured mutation yields has been scaled by the quantity in equation 6 and the resulting plot is expected to yield a straight line, independent of dose rate. In (b), the mutation yields have not been so scaled, but they still show a high correlation with survival which is independent of dose-rate.

#### 4. Discussion

In this paper, we have quantified an approach to understanding the observed inverse dose-rate effect for mutagenesis by sparsely ionizing radiation. The key assumption is that the effect is caused by a correlated variation in sensitivity across the cell cycle between mutagenesis and cell killing—there is quantitative evidence in this regard, from several experiments, that the correlation coefficient across the cell cycle between these two quantities is high.

We have quantified this approach using the LQR (Linear-Quadratic + Resensitization) formalism, which describes the response to a radiation-induced perturbation of a cycling, radiobiologically heterogeneous, population of cells. We have compared the model with dose- and dose-rate dependent mutagenesis data recently reported by Amundson and Chen (1996), and shown (Figures

2 and 3) that it can well reproduce the main features of the data, including the observed inverse dose-rate effect for mutagenesis.

Apart from the 'standard' linear-quadratic (LQ) parameters ( $\alpha_0$ ,  $\beta$ ,  $T_R$ ), the LQR model has two further key parameters, a resensitization amplitude describing the magnitude of the cell-cycle radiosensitivity variations, and a characteristic resensitization time for redistribution. The actual values of all five of the model parameters emerging from the fit to the mutation data are reasonable in magnitude, based on their biological interpretations.

In a key test, these five model parameters obtained from fitting the mutation data were used to predict the observed dose- and dose-rate dependent cell survival data—the heart of the model being that the same parameters should apply to both. The results were highly encouraging. Further internal tests of the assumed correlation between cell killing and mutagenesis (Figure 4) also add support to this approach.

The model predicts that the appearance of an inverse dose-rate effect, as opposed to a conventional sparing dose-rate effect, depends on the specific values of the radiobiological parameters for the cell line/end point of interest. Typical values of the parameters are such that either situation is quite possible, which would explain the apparently contradictory reports that have appeared in the literature for different mutation systems.

As we have discussed in the Introduction, there is a second class of explanations of inverse dose-rate effects, which do not require a cell-cycle-dependent correlation between the endpoint of interest and cell killing. The two classes of explanations have some similarities in that both are based on cell cycling, but the second class of explanation involves one or more ultrasensitive 'windows' in the cell cycle.

For mutagenesis, we suggest here that cell-cycle correlations represent a more likely explanation of the inverse dose-rate effect observed at low LET. Our reasoning is first, that the existence of a saturable intermediate damaged state underlying the process of mutagenesis, or of very short ultrasensitive 'windows' in the cell cycle, seem very unlikely. Second, the experimental data on variations through the cell cycle for mutagenesis and survival do generally show correlated radiosensitivities (Burki 1980, Jostes *et al.* 1980, Watanabe and Horikawa 1980, Chuang and Liber 1995) (Figure 1a). Finally, the present quantitative analysis suggests that the dose-rate dependent

mutation data analyzed here is both consistent with a correlation model for mutagenesis and cell killing, and is also internally consistent with the model assumptions.

It is interesting to note that this LQR correlation model implies that if there is an inverse dose-rate effect for cell killing then there will also be one for mutagenesis. However, the effect is predicted always to be smaller for cell killing than for mutagenesis, as is apparent from Figures 2 and 3. (Technically, this is because of the presence of the term  $\sigma^2 G_S D^2$  in the expression for mutation induction, compared with the term  $\frac{1}{2}\sigma^2 G_S D^2$  in the corresponding expression for survival.) Thus it may well happen that an inverse dose-rate effect is observed for mutagenesis, but that no such effect—or even a sparing effect—is observable for cell killing.

It is less clear whether such a correlation model is applicable to the inverse dose-rate effect observed at high LET for oncogenic transformation (Hill *et al.* 1982, Miller *et al.* 1988), and, by extension, carcinogenesis (Lubin *et al.* 1995), though such a suggestion has been made by Elkind (1994). The most complete experimental data (Miller *et al.* 1995) (Figure 1b) on the variation of sensitivity through the cell cycle of oncogenic transformation do not show variations that are correlated with cell killing; rather, the data show ‘windows of sensitivity’ for oncogenic transformation within the cell cycle. It is also noteworthy that a high-probability radiation-induced initial event in the oncogenic process (discussed above in relation to the ‘sensitive window’ explanation of the inverse dose-rate effect) has often been inferred (Kennedy 1985, Kamiya *et al.* 1995, Selvanayagam *et al.* 1995).

It is important to keep in mind that some inferences about the inverse dose-rate effect must be true irrespective of the underlying mechanisms. Specifically, at doses sufficiently low that at most a single track passes through the relevant target, no dose-rate effects of any kind would be expected (Barendsen 1985). This would hold whatever the correct model or models for the inverse dose-rate effect, and is of particular relevance for high-LET phenomena such as radon effects (Brenner 1994, Lubin *et al.* 1995).

In conclusion, we have presented arguments that the inverse dose-rate effects, which are sometimes observed in low-LET mutagenesis, are quantitatively understandable based on correlations across the cell cycle between cell killing and mutation induction. We have also suggested why the effect is not always observed. One interesting

outcome of this analysis is the implication that it is now possible to model, in a practical manner, the effects of radiation-induced cell-cycle perturbations. Our tool for this purpose, the LQR formalism, has only a small number of parameters, and it is likely that this relatively simple approach may be applicable in other similar contexts, such as cellular redistribution effects in radiotherapy.

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## A.1. Appendix

The formalism behind equations 1–3 is outlined here. We first describe the LQR formalism, which applies to the radiation response of a heterogeneous cell population, followed by a generalization to a system containing both mutated and non-mutated cells.

### A.2. The LQR equations

Consider a cell population which has subpopulations, such as cells at different stages of the cell cycle. Suppose the cell population is radiobiologically diverse because different subpopulations have different values of the LQ parameter  $\alpha$ , and that the proportion of cells in each subpopulation can change in time due, for example, to preferential killing or to cycling. Then the proportion of cells having a particular value of  $\alpha$  can also change in time, as can such quantities as the average of  $\alpha$  over the whole cell population. The LQR formalism models the time evolution of such a radiobiologically diverse cell population.

We will henceforth use the symbol  $\alpha_0$  for the average value over the whole cell population, when it is not perturbed by irradiation. For the average of a perturbed population, we will use  $\bar{\alpha}$ , and when considering different subpopulations, we shall simply use  $\alpha$ , to emphasize that many different values of this coefficient may be involved. For example, for a log-phase (i.e. exponentially cycling) cell population,  $\alpha_0$  is a constant which depends only on the particular cell-line, but many different values of  $\alpha$  are involved. Moreover, if the exponential cycling is perturbed by preferentially eliminating radiosensitive cells, the perturbed average,  $\bar{\alpha}$ ,

would be expected to decrease below the previous average value  $\alpha_0$ , at least temporarily.

We denote the cell number density in  $\alpha$  at time  $t$  by  $n(\alpha, t)$ , i.e.  $n(\alpha, t)d\alpha$  is the number of viable cells which have radiosensitivity in the small range  $\alpha$  to  $\alpha + d\alpha$ . In the absence of irradiation, the density,  $n$ , is taken to follow an Ornstein-Uhlenbeck diffusion process in the radiosensitivity space parameterized by  $\alpha$ , which tends to create a population structure with Gaussian diversity in  $\alpha$  (Bhattacharaya and Waymire 1990). Generalizations to the non-Gaussian case have been made (Brenner *et al.* 1995), but will not be used here.

It will be useful to denote by  $g(\alpha; \bar{\alpha})$  the Gaussian distribution having variance  $\sigma^2$  and mean  $\bar{\alpha}$ , i.e.

$$g(\alpha; \bar{\alpha}) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{(\alpha - \bar{\alpha})^2}{2\sigma^2}\right]. \quad (\text{A.1})$$

Let  $Ln$  be the Ornstein-Uhlenbeck diffusion operator  $L$  with parameters  $T_S$ ,  $\alpha_0$ , and  $\sigma^2$  acting on  $n(\alpha, t)$ , i.e.

$$Ln = (\partial n / \partial t) - (1/T_S) (\partial / \partial \alpha) [(\alpha - \alpha_0)n + \sigma^2 (\partial n / \partial \alpha)]. \quad (\text{A.2})$$

In the absence of irradiation, we use the Ornstein-Uhlenbeck partial differential equation with an additional growth term, i.e. we assume  $Ln = \lambda n$ , which implies that the total number of cells,  $N$ , grows exponentially in the absence of irradiation:

$$N(t) = N(0) \exp(\lambda t), \quad \text{where } N(t) = \int_{-\infty}^{\infty} n(\alpha, t) d\alpha.$$

$Ln = \lambda n$  also implies that there is a steady-state population structure, proportional to a Gaussian with mean  $\alpha_0$  and variance  $\sigma^2$ . Thus for  $t$  sufficiently large and no irradiation,  $n(\alpha, t) \rightarrow N(t)g(\alpha; \alpha_0)$ , where  $g$  is the function defined in equation A.1 (Bhattacharya and Waymire 1990). This steady-state pattern corresponds to the familiar pattern of cell number as a function of cell age in a log-phase cell population (Steel 1977), i.e. a pattern which has about half as many cells in M phase as in an equal age window near the start of the G<sub>1</sub> phase, the main difference in the present formalism being that sensitivity ( $\alpha$ ) replaces age.

In equation A.2,  $T_S$  is interpreted as a characteristic resensitization time. Roughly speaking, this means that if the average population radiosensitivity,  $\bar{\alpha}$ , at one instant deviates from the average value,  $\alpha_0$ , for the steady-state population structure there is a tendency for  $\bar{\alpha}$  to decay exponentially back to  $\alpha_0$ , with time constant  $T_S$  (Hlatky *et al.* 1994). This tendency is modulated by diffusion

effects, corresponding to a loss of cell-cycle synchrony. The background here is that detailed models of cycling cell populations (e.g. Hahnfeldt and Hlatky 1996) do show an overall tendency to restore the average radiosensitivity after a perturbation, so in a general cell population which has many kinds of subpopulations, it is useful to approximate this restoring tendency by a simple exponential decay of the excess or deficit in the average.

Now suppose the population is irradiated with a time-dependent dose rate  $\dot{D}(t)$ . We shall suppose irradiation occurs within the finite time interval  $[0, T]$ , so that

$$D = \int_0^T \dot{D}(t) dt,$$

where  $D$  is the total dose. The underlying equations of the LQR model are

$$\begin{aligned} (\text{A}) \quad & du/dt = c\dot{D} - (u/T_R) - 2Ku^2, \\ (\text{B}) \quad & Ln = \lambda n - F(\alpha, t)n, \\ (\text{C}) \quad & n(\alpha, 0) = N(0)g(\alpha; \alpha_0); \end{aligned} \quad (\text{A.3})$$

where

$$F(\alpha, t) = \alpha \dot{D}(t) + \frac{1}{2}Ku^2(t). \quad (\text{A.4})$$

The interpretation of these equations is as follows:

- (A)  $u(t)$  is the average number of sublethal lesions per cell at time  $t$ . The time development of  $u$  consists of  $c\dot{D}$ , corresponding to creation of sublethal lesions at a rate proportional to dose delivery,  $-u/T_R$  corresponding to repair at rate  $1/T_R$ , and  $-2Ku^2$ , corresponding to quadratic misrepair at rate  $K$ . This equation is similar to that in the LPL model (Curtis 1986).
- (B) Equation A.3B reduces to the Ornstein-Uhlenbeck equation discussed above, if the dose-rate is zero and there are no sublethal lesions. Otherwise there is an extra term,  $F_n$ , interpreted as implying that cells are removed from the viable population at a per-cell rate of  $\alpha \dot{D}$  for one-track action and at a rate  $\frac{1}{2}Ku^2$  for binary misrepair.
- (C) Equation A.3C states that, just prior to the start of irradiation, the population is in the steady-state Gaussian pattern, proportional to  $g$  in equation A.1.

We will here use the approximation (Curtis 1986, Thames and Hendry 1987) that repair of sublethal lesions dominates misrepair, i.e. that in

equation A.3A, though not in equation A.3B, the term involving  $K$  can be neglected. This is reasonable in view of estimates that 1 Gy of sparsely ionizing radiation typically produces about 40 DNA double-strand breaks, but only one fatal misrepair (Sachs and Brenner 1993).

For any arbitrary dose-rate, a calculation using equations A.3 and A.4 (Hlatky *et al.* 1994, Hahnfeldt and Sachs 1996) gives the following expression for log-surviving fraction at time  $T$ , valid for any dose-rate pattern:

$$\ln S = -\alpha_0 D - \beta G_R D^2 + \frac{1}{2} \sigma^2 G_S D^2 + \lambda T, \quad (\text{A.5})$$

where  $\beta = T_R K c^2 / 4$ . This is equation 1 in the main text. Here  $G_x$  ( $x = R$  or  $S$ , referring respectively to repair/misrepair or resensitization) is the generalized Lea-Catcheside function (Brenner *et al.* 1991), i.e.

$$G_x = 2 \int_0^T f(t) dt \int_0^t \exp\left[\frac{-(t-t')}{T_x}\right] f(t') dt',$$

with  $f(t) = \dot{D}(t)/D$ . (A.6)

Equation A.5 models the combined effects of one-track damage ( $\alpha$ ), repair or misrepair ( $\beta$ ), proliferation ( $\lambda$ ) and resensitization ( $\sigma^2$ ). As a special case, for a constant uniform dose rate during the time interval  $[0, T]$  and zero dose otherwise, integrating in equation A.6 gives equation 2 of the main text.

### A.3. Model for mutagenesis

Suppose now there is a second, mutated population, having the same cell cycle kinetics and radiation response as the population of non-mutated cells. Suppose further that there is perfect correlation between killing and mutagenesis, i.e. a fixed, very small fraction,  $\varepsilon$ , of 'killed' non-mutated cells was actually removed from the non-mutated population by mutagenesis at a particular locus, rather than by being rendered non-viable. Let  $\mu(\alpha, t)$  denote the population density of mutated cells at time  $t$ . Then, with the non-mutated population again described by a function  $n(\alpha, t)$  obeying equations A.3 and A.4,  $\mu$  obeys

$$L\mu = \lambda\mu - F\mu + \varepsilon F n; \quad \mu(\alpha, 0) = 0. \quad (\text{A.7})$$

$L$  is defined in equation A.2 and  $F$  in equation A.4. The fact that  $L$ ,  $\lambda$  and  $F$  again appear in the first three terms of equation A.7 follows from the assumption that the mutated cells have the same kinetics and radiosensitivity as the non-mutated

ones. The term  $\varepsilon F n$  in equation A.7 is a creation rate for mutated cells, and the appearance, once more, of the same function  $F$  corresponds to the assumption, discussed in the Introduction and Methods sections, that mutagenesis and killing are correlated.

Equations A.3, A.4 and A.7 mathematically define the LQR correlation model for mutagenesis. The solutions for the special case  $\lambda = 0$ , when only the usual Ornstein-Uhlenbeck differential operator,  $L$ , is involved, have been described elsewhere (Hahnfeldt and Sachs 1996). The case  $\lambda \neq 0$  can be reduced to the case  $\lambda = 0$  by the following technique: Temporarily define auxiliary densities by factoring out the exponential proliferation rate, i.e.  $\tilde{n} = n \exp(-\lambda t)$  and  $\tilde{\mu} = \mu \exp(-\lambda t)$ . Upon substituting the auxiliary densities,  $\tilde{n}$  and  $\tilde{\mu}$ , into equations A.3, A.4 and A.7, a short calculation shows that these auxiliary densities also obey the same equations, but with  $\lambda = 0$ . Therefore, the known solutions can be used for  $\tilde{n}$  and  $\tilde{\mu}$ , thereby also obtaining  $n = \exp(\lambda t)\tilde{n}$  and  $\mu = \exp(\lambda t)\tilde{\mu}$ . Then we can compute the fraction,  $M$ , of mutated surviving cells, defined mathematically by

$$M = \lim_{t \rightarrow \infty} \frac{\int_{-\infty}^{\infty} \mu(\alpha, t) d\alpha}{\int_{-\infty}^{\infty} n(\alpha, t) d\alpha}. \quad (\text{A.8})$$

Taking the limit in equation A.8 by using the solutions  $n$  and  $\mu$ , obtained as described above, we obtain, after a further calculation,

$$M = \varepsilon [\alpha_0 D + \beta G_R D^2 - \sigma^2 G_S D^2], \quad (\text{A.9})$$

which is equation 3 of the main text, interpreted in the Methods section, and used in the Results section for fitting the experimental data. It should be noted that, due to the idealizations involved in the model, the domain of validity of this equation is within the region where  $M$  is positive (Hlatky *et al.* 1994). Equation A.9 indicates that  $M$  is independent of the cellular growth rate,  $\lambda$ , which is because we are calculating a ratio of mutated to non-mutated cells, where both populations are growing with the same kinetics.

It is interesting to note that, while equation A.9 for mutation yield per surviving cell is structurally similar to equation A.4 for cellular survival, it is not identical. Specifically, the expression for survival contains the term  $\frac{1}{2} \sigma^2 G_S D^2$ , whilst the expression for mutation yield per surviving cell contains the term  $\sigma^2 G_S D^2$ , differing by a factor of  $\frac{1}{2}$ .

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