Review Article

The dose-rate effect in human tumour cells

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Summary

The radiation response of 12 cell lines derived from a variety of human tumours has been investigated over the dose-rate range from 150 to 1.6 cGy/min. As the dose rate was lowered, the amount of sparing varied widely; in 2 cell lines it was zero, in the other cell lines the dose required for 10^{-2} survival ranged up to twice the value at high dose rate. Low dose-rate irradiation discriminates better than high dose rate between tumour cell lines of differing radiosensitivity. The data are equally well fitted by two mathematical models of the dose-rate effect: the LPL model of Curtis and the Incomplete Repair model of Thames. Analysis by the LPL model leads to the conclusion that the theoretical radiosensitivity in the total absence of repair was rather similar among the 7 cell lines on which this analysis was possible. What differs among these cell lines is the extent of repair and/or the probability of direct infliction of a non-repairable lesion. Recovery from radiation damage was also examined by split-dose experiments in a total of 17 human tumour cell lines. Half-time values ranged from 0.36 to 2.3 h and there was a systematic tendency for split-dose halving times to be longer than those derived from analysis of the dose-rate effect. This could imply that cellular recovery is a two-component process, low dose-rate sparing being dominated by the faster component. The extent of low dose-rate sparing shows some tendency to correlate with the magnitude of split-dose recovery; in our view the former is the more reliable measure of cellular recovery. The clinical implication of these studies is that some human tumour types may be well treated by hyperfractionation or low dose-rate irradiation, while for others these may be poor therapeutic strategies.

Introduction

It could be said that the dominant factor on which success or failure in clinical radiotherapy depends is recovery from radiation damage. Splitting a given total dose into many fractions allows repair after each fraction and the total recovered dose may thus be considerable. In skin, recovery after 30 fractions can amount to 150-200% of the single-dose level [5] and together with cellular repopulation is the principal reason why fractionation is so widely used
in clinical radiotherapy. But recovery also protects tumour cells. It seems likely that those tumour types that are controlled by fractionated radiotherapy are less capable of repairing damage than cells in the limiting normal tissues; conversely, a major reason for failure is that tumour cells recover to a similar degree as the normal tissue stem cells. Current interest in the clinical use of small fraction sizes, given in multiple fractions per day, derives from the experimental observation that the late-reacting normal tissues are relatively spared by lowering the dose per fraction, and this must be due to their greater ability to recover from radiation damage.

Low dose-rate irradiation is the limiting case of increased fraction number and reduced dose per fraction. It allows maximal recovery from radiation damage, for a given duration of treatment, and the lower the dose rate the greater the opportunity for recovery.

The clinical use of low dose-rate radiotherapy has a long history. It has most commonly been used in interstitial or intracavitary treatment but since these treatments have mainly been designed to exploit the geometric advantages of the very non-uniform fields around radioactive sources, it has been difficult to deduce whether the low dose rate by itself carries a therapeutic advantage [9].

This paper summarizes the results of a research programme that was designed to investigate the dose-rate effect in human tumour cells from two points of view: as a means of learning more about cellular recovery processes in tumour cells and as a way of throwing light on the therapeutic benefits of low dose-rate irradiation as used clinically. The data on individual tumour types have been or will be published in detail elsewhere; the present review examines the broad features of the dose-rate effect in the human tumour cell lines that we have employed. It also examines the data in the light of two recently-described models of dose-rate-dependent cell survival: the Lethal-Potentially Lethal model of Curtis [1] and the Incomplete Repair model of Thames [15].

**Biological mechanisms underlying the dose-rate effect**

Clinical radiation treatments are usually given within a few minutes. If the treatment time is prolonged by reducing the dose rate, a number of biological processes are permitted to take place during treatment, thus modifying the radiation effect. Four processes are of particular importance, the 4 Rs of radiobiology: Repair, Reassortment, Reoxygenation, Repopulation [17].

The range of dose rates over which any of these processes will modify response depends on its speed. Repair is the fastest, associated with a half-time of perhaps an hour or less; reassortment will occur within the approximate duration of the cell-cycle phases (a few hours); repopulation requires one or more cell-cycle times (a few days in human tumour cells); the speed of reoxygenation in human tumours is difficult to specify but it may well vary over the range described for reassortment and repopulation. Any particular process will modify response whenever the duration of treatment becomes comparable with the half-time of the process. Fast processes (short half-times) will be able to compete with a rapid infliction of damage (high dose rate). Conversely, a slow process will only influence response at low dose rate.

The irradiation time for a dose of 2 Gy is 2 min at 100 cGy/min, 20 min at 10 cGy/min, and 200 min (i.e. 3.3 h) at 1 cGy/min. As we have argued previously [12] the dose-rate effect down to about 2 cGy/min will therefore be dominated by repair processes. At around 2 cGy/min cell-cycle progression phenomena may begin to modify response. Nevertheless, studies of the sparing effect of reducing the dose rate from say 100 to 2 cGy/min can give information on the kinetics of cellular recovery from radiation damage.

**The radiation response of 17 human tumour cell lines**

During the past few years, we have made detailed radiobiological studies on over 17 xenografts and cell lines derived from a variety of human tumours.
In 12 of these we have examined the dose-rate effect. Table I lists the tumour lines and gives the parameters of the acute radiation survival curves (dose rate 1–2 Gy/min). All of these acute curves are well fitted by the linear-quadratic equation:

\[ S = \exp(-\alpha D - \beta D^2) \]

where \( D \) is dose and \( \alpha \) and \( \beta \) are constants.

In terms of our recent classification of radioresponsiveness in human tumours [2] this whole group of tumour lines covers the full range from the highly responsive neuroblastomas (Group A) to the unresponsive melanomas (Group E). The surviving fractions at 2 Gy range from 0.08 to 0.82 and the ranking of radiosensitivity is broadly in keeping with what we might expect from these tumour types. The main anomaly is the bladder line WX67 which has a shoulderless survival curve and is much more radiosensitive than the other bladder line RT112. It may be noted that WX67, HX138, HX143, HX144 and HX149 all have \( \alpha/\beta \) ratios in excess of 20; these curves are indistinguishable from exponential.

Most of the cell lines were derived from tumours that had first been xenografted into immune-deficient mice. The exceptions were HX156, HX149, HC12, and GCT27, which were established directly in tissue culture. Radiobiological experiments were performed on single-cell suspensions prepared by enzymatic disaggregation of xenografts or confluent monolayers. In all cases, a standard pre-, during, and post-irradiation protocol was then followed. Cells were plated out at appropriate density in soft agar culture or on plastic in monolayer, and held for 2 h at 37°C in a 5% \( \text{O}_2 \), 5% \( \text{CO}_2 \), 90%

<table>
<thead>
<tr>
<th>Acute survival curve</th>
<th>Split-dose exponential</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>( \beta )</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>HX34 Melanoma</td>
<td>0.32</td>
</tr>
<tr>
<td>HX118 Melanoma</td>
<td>0.36</td>
</tr>
<tr>
<td>HX32K Pancreas</td>
<td>0.42</td>
</tr>
<tr>
<td>HX58 Pancreas</td>
<td>0.66</td>
</tr>
<tr>
<td>HX99 Breast</td>
<td>0.20</td>
</tr>
<tr>
<td>HX156 Cervix</td>
<td>0.20</td>
</tr>
<tr>
<td>WX67 Bladder</td>
<td>1.18</td>
</tr>
<tr>
<td>HX144 Lung adenocarcinoma</td>
<td>0.44</td>
</tr>
<tr>
<td>HX148 Lung adenocarcinoma</td>
<td>0.32</td>
</tr>
<tr>
<td>HX147 Lung LC</td>
<td>0.056</td>
</tr>
<tr>
<td>HC12 Lung SC</td>
<td>0.43</td>
</tr>
<tr>
<td>HX149 Lung SC</td>
<td>0.63</td>
</tr>
<tr>
<td>RT112 Bladder</td>
<td>0.10</td>
</tr>
<tr>
<td>GCT27 Teratoma</td>
<td>0.37</td>
</tr>
<tr>
<td>HX138 Neuroblastoma</td>
<td>1.08</td>
</tr>
<tr>
<td>HX142 Neuroblastoma</td>
<td>0.84</td>
</tr>
<tr>
<td>HX143 Neuroblastoma</td>
<td>1.16</td>
</tr>
</tbody>
</table>

\( ^a \) Surviving fraction at 2 Gy.

\( ^b \) Dose per fraction (Gy).

\( ^e \) Parameter of split-dose recovery: \( A = \) time-zero survival level; \( R = \) split-dose recovery ratio; \( T_{1/2} = \) half-time for split-dose recovery (h).

\( ^d \) ABRR is the recovery ratio calculated as \( \exp(2/\beta d^2) \).
The gaseous environment before irradiation with $^{60}$Co γ-rays at the appropriate dose rate. Cells were maintained at 37°C and in the low O$_2$ atmosphere throughout irradiation and during the post-irradiation period of colony growth.

The acute radiation survival curves of the 12 cell lines on which we have dose-rate data are summarised in Fig. 1A. Although there is a range of radiosensitivity, it can be seen that the final slopes of these acute curves do not vary widely (in fact by a factor of 2.0). The major difference is in the steepness of the initial slope (the α parameters differ by a factor of up to 12).

Figure 1B shows the corresponding range of radiosensitivity at low dose rate (1.6 cGy/min). At this dose rate most, but not all, of the survival curves have become exponential. The curves that were steepest at high dose rate have not moved much but as expected the survival curves for the more resistant lines have flattened considerably. The final slopes now differ by a factor of 7.0. It can be seen that low dose-rate irradiation discriminates better than high dose rate between tumour cell lines of differing radiosensitivity. The relationship between high- and low dose-rate sensitivity is shown in Fig. 2. From each of the sets of data in Fig. 1, we have calculated (by linear-quadratic interpolation) the dose required to achieve $10^{-2}$ survival either at high dose rate (150 cGy/min) or low dose rate (1.6 cGy/min). A smooth curve can be drawn through these values, showing that as the acute radiosensitivity decreases (acute $D_{0.01}$ value increasing) there is a progressive tendency for the dose-rate effect to become more pronounced. When the acute $D_{0.01}$ is 5 Gy the ratio is 1.24; when it is 10 Gy the ratio is 2.0.

**Simulation of cell-survival data using the LPL model**

Most models of radiation cell survival allow the calculation of response as a function of dose but do
not take account of dose rate. It is quite difficult to adapt the multitarget or linear-quadratic equations in a rational way to deal with changes in dose rate. Oliver [11] suggested an empirical way of doing this which envisaged that the “dose-equivalent” of recoverable damage should decay exponentially. This approach has more recently been used by Thames [15] in his “Incomplete Repair” model.

The Lethal-Potentially Lethal (LPL) model of Curtis [1] is conceptually different. It is a kinetic model of radiation cell killing in which two types of lesion are thought to occur: lethal (irrepairable) lesions, and potentially lethal lesions. The potentially lethal lesions may either be repaired to restore cell viability, or misrepaired and thus converted into lethal lesions (they are thus “fixed”). It is envis-aged that any one unrepaired lesion (of either type) will lead to cell death.

The model has four main parameters:

- \( \eta_l \) — the probability per unit dose of directly inducing a lethal lesion
- \( \eta_{pl} \) — the probability per unit dose of inducing a potentially lethal lesion
- \( \varepsilon_{pl} \) — the rate constant for repair of potentially lethal lesions
- \( \varepsilon_{2pl} \) — the rate constant for misrepair of potentially lethal lesions.

The designation \( \varepsilon_{2pl} \) reflects the assumption in the model that misrepair is associated with the interaction of two potentially lethal lesions. It is this process that leads to downward-bending survival curves as dose is increased.

In our computer formulation of this model we have preferred to work in terms of more easily visualised functions of these four parameters:

\[
D_S = \frac{1}{\eta_l} \quad \text{(determines the slope of the fully repaired survival curve)}
\]

\[
D_0 = \frac{1}{\eta_l + \eta_{pl}} \quad \text{(determines the slope of the totally unrepaired survival curve, line B in Fig. 3)}
\]

\[
T_{1/2} = \frac{\log_2 e}{\varepsilon_{pl}} \quad \text{(the half-time for repair)}
\]

\[
E = \frac{\varepsilon_{pl}}{\varepsilon_{2pl}} \quad \text{(the ratio of rate constants for repair/misrepair)}.
\]

A fifth variable in the model is the time during which repair continues to occur after the end of irradiation and during the cell cloning procedure. In the experiments reported here the cells were disaggregated, plated, and then irradiated. Throughout the present calculations this parameter has therefore been given a fixed value of 5 h, a value that is more than 5 times the longest repair half-time that we have determined.

The LPL model produces cell-survival curves that are almost identical to the linear-quadratic equation down to survival levels of about \( 10^{-2} \).
Below this level, the linear-quadratic curve bends continuously while the LPL model gives exponential cell kill at high doses.

This model has been fitted to cell-survival data by minimising the sum of the squares of deviations of experimental points from the calculated curves. Deviations were measured as log(survival) and the minimisation was performed using a programme provided by Dr. K. Koschel.

An example of data fitted in this way is shown in Fig. 3 (from ref. [10]). There are data at 3 dose rates: 150, 7.6 and 1.6 cGy/min. Considerable dose-rate sparing is observed in this human melanoma and at the lowest dose rate the survival curve has become almost exponential. The broken lines indicate calculations on the basis of the fitted LPL model of the position of the survival curves where repair is complete (curve A, corresponding to infinitely low dose rate) and where no repair occurs (curve B). The curve calculated for 150 cGy/min is close to that which would be obtained at infinitely high dose rate; its curvature and displacement from B reflect “unstopable recovery”, i.e. recovery that is envis-

![Cell-survival curves for a human melanoma cell line (HX118) irradiated at 150, 7.6, or 1.6 cGy/min. The data are fitted by the LPL model from which we derive the survival curve where repair is complete (curve A) or where repair is totally absent (curve B).](image)

**TABLE II**

Parameter of the LPL and IR models fitted to cell-survival data.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Curtis LPL model</th>
<th>Thames IR model</th>
<th>DRF&lt;sup&gt;b&lt;/sup&gt;&lt;sub&gt;150-1.6&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>$D_s$</td>
<td>$D_0$</td>
</tr>
<tr>
<td>HX34</td>
<td>4</td>
<td>3.7</td>
<td>0.48</td>
</tr>
<tr>
<td>HX118</td>
<td>3</td>
<td>3.1</td>
<td>0.77</td>
</tr>
<tr>
<td>HX32K</td>
<td>2</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>HX58</td>
<td>2</td>
<td>2.2</td>
<td>0.28</td>
</tr>
<tr>
<td>HX99</td>
<td>3</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>HX156</td>
<td>3</td>
<td>3.3</td>
<td>0.32</td>
</tr>
<tr>
<td>WX67</td>
<td>5</td>
<td>0.85</td>
<td>e</td>
</tr>
<tr>
<td>RT112</td>
<td>7</td>
<td>9.8</td>
<td>0.57</td>
</tr>
<tr>
<td>GCT27</td>
<td>3</td>
<td>2.2</td>
<td>0.40</td>
</tr>
<tr>
<td>HX138</td>
<td>2</td>
<td>c</td>
<td>c</td>
</tr>
<tr>
<td>HX142</td>
<td>2</td>
<td>8.8</td>
<td>0.54</td>
</tr>
<tr>
<td>HX143</td>
<td>2</td>
<td>0.76</td>
<td>e</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of dose-rate levels at which data were available.

<sup>b</sup> Dose Reduction Factor (150–1.6 cGy/min) calculated from actual data.

<sup>c</sup> Data incompatible with LPL and IR models.

<sup>d</sup> Half-time constrained to the LPL value.

<sup>e</sup> No significant dose-rate effect.

Units as follows: $D$, Gy<sup>-1</sup>, $D_0$, Gy<sup>-1</sup>, $T_{1/2}$, hours.
Fig. 4. Low dose-rate sparing as a function of dose rate in human cell lines. DRF indicates the dose required at the specified dose rate to produce $10^{-2}$ survival divided by the dose to produce this effect at infinitely high dose rate. (A) For a selected group of human cell lines, the response calculated from the LPL model fitted to the data. (B) Results derived directly from cell-survival curves.

The sparing effect of low dose-rate irradiation can usefully be described by the type of representation given in Fig. 4. For each cell line and at each dose rate we have determined the radiation dose required to give 1% survival (the $D_{0.01}$ value [12]). We have done this either by calculation on the basis of the LPL model, or by fitting each data set individually with a linear-quadratic equation and thus interpolating the $D_{0.01}$ value. The model calculation can be performed at any dose rate, and it yields the continuous curves shown in Fig. 4A. The Dose Reduction Factor (DRF) indicates the ratio: $D_{0.01}$ at specified dose rate/$D_{0.01}$ at high dose rate. We use the term Dose Reduction Factor by analogy with Dose Enhancement Factor or Sensitiser Enhancement Ratio that are used when there is a change towards steeper survival curves.

In interpreting these curves it must be remembered that the two models deal only with changes in radiation response that results from recovery from radiation damage: cell cycle progression and proliferation are ignored. One interesting feature is that the two models predict that repair influences response over as much as a 100-fold range of dose rates. In the case of HX34, the DRF reaches 1.1 at 80 cGy/min but the curve is still not completely flat at 1 cGy/min. In model calculations the dose rate corresponding to the mid-point of this rise correlates well with the calculated half-time for recovery.

DRF values have also been calculated from the actual data sets at each dose rate and these are plotted in Fig. 4B. The reference value for $D_{0.01}$ was taken as that predicted by the LPL model at high dose rate. The trajectories in this diagram indicate the extent of low dose-rate sparing directly observed (i.e without possible distortion as a result of the model-fitting procedure). There clearly is a wide
range among these cell lines in the amount of sparing. We have quantified this by measuring the slope of these lines: the DRF value that corresponds to a dose-rate change from 150 to 1.6 cGy/min is termed the DRF\textsubscript{150-1.6} [12]. We choose this range of dose rates over which to measure DRF because it corresponds to the maximum range that we have investigated and because it gives a good indication of the amount of sparing that would be associated with clinical interstitial therapy. The resulting values are given in Table II. They range from 1.0 in HX143 to 2.1 in HX34 and HX112, with a median of 1.4.

**Comparison with split-dose recovery kinetics**

Most of the cell lines that are the basis of this review have also been investigated using split-dose experiments as an independent way of obtaining information on the extent and kinetics of recovery from radiation damage. Two equal single doses were given, under in vitro irradiation conditions that were the same as those used for low dose-rate irradiation; the dose levels were chosen to give a maximal (i.e. simultaneous dose) effect of about 2 logs of cell kill. Split-dose intervals up to about 6 h were investigated.

The split-dose results were analysed by fitting a simple rising exponential to the surviving fraction data:

\[ S = A \cdot R^{1 - e^{-\mu t}} \]

where \( A \) = intercept at zero time; \( R \) = recovery ratio; \( \mu \) = time constant for recovery = \( \log_2(T_{1/2}) \), and \( e = \exp(-\mu t) \).

The results of this analysis are given in Table I. Values for recovery ratio varied from 1.2 to 3.3, and the half-times for recovery showed a 6-fold variation (from 0.36 to 2.3 h).

As might be expected, there is a tendency for split-dose recovery to correlate with the acute radiosensitivity of the cell lines: the more resistant lines show greater recovery. This can be seen in Fig. 5A where the experimentally-determined recovery ratios (\( R \) in Table I) are plotted against the dose required for two decades of cell kill at high dose rate. There is a fair degree of scatter in this plot, part of which is attributable to the fact that in lines

![Fig. 5. Correlation between recovery ratio (\( R \) in Table I) and the high dose-rate radiosensitivity (\( D_{0.01} \), A) or low dose-rate sparing (DRF\textsubscript{150-1.6}, B). The circled numbers are abbreviated cell line numbers.]
that have a large shoulder (RT112 in particular) the individual doses used in the split-dose experiments were well on the shoulder of the acute curve. In these lines, a higher dose per fraction should have given a considerably higher recovery ratio. We have not attempted to correct for this effect, for reasons that will be set out in the Discussion.

For 11 cell lines it is possible to examine the relationship between low dose-rate sparing and split-dose recovery (Fig. 5B). There is a suggestion of a positive correlation but the level of confidence in this is not high (correlation coef. = 0.52). The reason for the low point at DRF = 2.1 (RT112) has been explained above.

For 6 cell lines we are able to compare the estimates for recovery half-time obtained either by analysing dose-rate dependence or the split-dose recovery. The results are shown graphically in Fig. 6. There are 9 split-dose half-times in Table I for which we have no corresponding dose-rate values; their range (mean 1.27, S.D. 0.72) is similar to those plotted in Fig. 6. The half-times from dose-rate analysis are always shorter than from split-dose experiments, by a factor of 10 in the case of HX34, 3 in HX142 and less than 2 in the other three lines.

Discussion

This summary of cell-survival data on human tumour cell lines has described the range of responses observed at high and low dose rate. Figure 1 clearly contradicts the view sometimes expressed that radiation is a relatively non-specific killing agent. It is true that the acute $D_0$ values do not vary greatly, but this statement fails to reflect the wide differences in shoulder size that are shown in the Fig. 1A. Irradiation at a dose rate that allows considerable opportunity for repair without allowing much cell cycle progression gives the results shown in Fig. 1B. The doses required to give a fixed level of cell kill now differ by a factor of 7 and as shown in Fig. 2 they correlate well with the high dose-rate radiosensitivity.

The range of sensitivities at low dose rate may well reflect what would be observed clinically. Treatment with 2 Gy fractions, allowing recovery between doses, would be expected to produce survival curves that extrapolate the slope of the acute curves from the origin to the 2 Gy survival point and these would be very similar to the curves shown in Fig. 1B. We can calculate from them the levels of cell kill that would be achieved by a total dose of say 50 Gy (see a similar calculation by Withers [16]). Some of these values depend very much on whether one assumes that the low dose-rate survival curves continue to follow a linear-quadratic equation down to very low survival levels. If we assume not, then the calculated surviving fraction depends only on the $\alpha$ component of the low dose rate survival curves and we obtain the following estimates of cell kill at 50 Gy:

HX34, HX118, HX156, RT112: $10^{-4}$ to $10^{-6}$
HX99, HX58, GCT27, HX142: $10^{-8}$ to $10^{-12}$
HX32, WX67, HX143, HX138: $10^{-15}$ to $10^{-20}$.

These are of course only very rough estimates of the expected clinical result but they do show that the family of low dose-rate curves presented in Fig. 1 covers a range of sensitivities that could easily explain widely differing probabilities of local control.
The two models (LPL and IR) that we have used to simulate the dose-rate effect do so equally satisfactorily. The LPL model has the advantage of being based on plausible intracellular mechanisms of repair and misrepair of radiation damage, and their associated time constants. Unfortunately, this conceptual advantage does not lead us to define these mechanisms with useful precision. The LPL model is described by 4 parameters (5 in fact, see above) and the experimental data that we have been able to obtain do not fix these parameters very accurately. We have found situations in which the $E$ and $D_0$ parameters can be traded off against one another, with little effect on the quality of fit. We have also found that data are required at least at 3 dose rates in order to obtain a reliable fit. The constraints on the LPL and IR models are somewhat different and we have concluded that only when they arrive at closely similar conclusions (as they always do when there is plenty of data) can the results be regarded as reliable.

If the objective is to determine the half-time for repair, it would seem that either model will give this. Of the 12 cell lines on which we have data at two or more dose rates there are only 7 on which the data are adequate to determine reliable values for recovery half-time. In two lines (HX143 and WX67) there was no significant dose-rate effect and therefore the half-time is not defined. In 3 further lines (HX32K, HX99, HX138) the data were for one reason or another incompatible with the LPL and IR models and we have therefore not attempted to quote parameter values. The resulting 7 analyzed cell lines give half-time values ranging from 0.1 to 0.85 h. As indicated in Fig. 6, these values are systematically shorter than estimates of half-time for recovery derived on the same cell lines from split-dose experiments. HX34 is an extreme case, with a split-dose half-time that is a factor of 10 greater than the dose-rate value. For the other 5 lines on which we can make this comparison (we do not have split-dose data on HX156) the factor varies from 1.1 to 3.0. Excluding HX34, the mean value for this factor is 1.8.

Why should these two experiments give such different estimates for repair half-time? We are inclined to postulate that recovery from radiation damage involves a group of processes that have different time constants. Thus, although recovery can usually be described by a single exponential it could in reality have two or more components. The split-dose recovery analysis will tend to average these and be dominated by the slower component. The dose-rate analysis, depending as it does on rate constants for recovery (and infinitely small dose increments) may tend to be dominated by the faster component. Recovery curves of this type have not usually been suggested by split-dose recovery data, but this could be because split-dose experiments (especially on normal tissues) have often used relatively large doses and exposure times of 10 min or more. Such exposures would probably fail to demonstrate an initial fast recovery component. However, studies of the time-course of rejoicing of DNA strand breaks have shown non-exponential kinetics. Dikomey and Franzke [4] were able to extract 3 components in the rejoicing curves for CHO cells, whose half-times were 0.03, 0.3 and 2.8 h. This range spans the half-times that we have calculated. This could be fortuitous, especially since the fidelity of strand rejoicing may differ between the rapidly-rejoined (presumably single-strand) breaks and the slowly-rejoined (perhaps double-strand) breaks.

Amongst the 7 cell lines for which we have adequate data the LPL model gives $D_0$ values ranging from 0.28 to 0.77 Gy with a mean of 0.48 Gy. The model implies therefore that among these human tumour cell lines this would be the level of radiation sensitivity in the total absence of repair. Similar values of $D_0$ appear to apply not only to the highly radiosensitive neuroblastomas but also to the relatively radioresistant HX34, HX156 and RT112 cell lines. Thus, on this model the reasons for radioresistance lie either in a high $D_s$ value (i.e. cells insensitive to direct lethal events) or in a high value of $E$ (most potentially lethal damage is repaired rather than fixed). It is disappointing that the reasons for resistance cannot be attributed to one or other of these very different processes.

Our data allow us to compare three different indicators of radiation damage repair: the size of the shoulder on the acute cell survival curve, the extent
of split-dose recovery, and the extent of low dose-rate sparing. Since our acute survival curves appear to conform to the linear-quadratic equation, the shoulder size cannot be specified directly. But, as shown by Thames [15] the linear-quadratic equation leads to a simple expression for split-dose recovery ratio:

Theoretical recovery ratio = \( \exp(2\beta d^2) \)

where \( \beta \) is the coefficient of the dose-squared term and \( d \) is the dose per fraction. We have calculated values using this relation (ABRR in Table I). For most of our lines, the correspondence with the actual recovery ratios (\( R \)) is quite good. However, in the case of three lung carcinoma lines (RT144, 148 and HC12) ABRR greatly underestimates the observed recovery and in the case of the HX112 bladder carcinoma line (which has a large shoulder and low \( \alpha/\beta \) ratio) it gives a considerable overestimate. The scatter in these results may be due to the fact that recovery ratio in the equation given above depends steeply on the value of \( \beta \) (which is often poorly defined by cell survival data) and even more so on the value of \( d \). This may also explain why the correlations between recovery ratio (\( R \)) and the high dose-rate sensitivity (Fig. 5A) and DRF value (Fig. 5B) are not better.

One of our objectives in this work has been to classify a group of human tumour cell lines in terms of their ability to recover from radiation damage. Our work is now moving towards the investigation of molecular mechanisms in the radiation response of human tumours and we wish to relate the results of such studies to parameters of cellular radiobiology. Which is the most reliable measure of the capacity for cellular recovery after irradiation? From our comparison of recovery ratio, low dose-rate sparing, and the ABRR factor, we are inclined to the view that of these the low dose-rate sparing factor is the most reliable. When the acute dose-response curve is continuously bending the actual and theoretical recovery ratios may be a steep function of the chosen dose level; if the curve shape differs from one cell line to another, at what dose levels should we compare recovery ratios? The DRF factor as we have defined it not only seems to provide a more robust basis of comparison between cell lines, but it is also clinically appropriate. The low dose-rate survival curves shown in Fig. 1 probably reflect the levels of cell kill that might be expected from a short course of fractionated radiotherapy and the DRF values indicate the amount of cellular recovery that is associated with this dose-rate effect.

The extent of low dose-rate sparing is of direct clinical interest. Although interstitial radiotherapy mainly exploits the geometrical advantages of irradiation from within the tumour, its success may well depend upon whether the low dose rates that are employed lead to a therapeutic advantage or disadvantage by comparison with more conventional dose rates. The cell lines that we have studied here vary widely in the extent of sparing, as can be seen from the data in Fig. 4B and the calculated values for \( \text{DRF}_{150-1.6} \) (Table II). In a previous review of low dose-rate sparing in murine normal tissues [12] we found a similar range of sparing factors. It is therefore not possible to claim that in general the use of low dose rate in clinical radiotherapy should lead to a therapeutic advantage. It could do so if there is a tendency for the target cells for late normal tissue damage to have greater repair capacity (and more curvy dose-response curves) than we have found here. One might be led to expect this from recent analysis of fractionation studies [8,14] but few direct low dose-rate studies on late-reacting normal tissues have so far been done. The amount of low dose-rate sparing of pneumonia in the mouse lung is certainly considerable [6].

At the present time, the most obvious conclusion to be drawn from the wide range of sparing seen in these human tumour cell lines is that depending upon the precise circumstances a lowering of dose rate could either lead to a therapeutic advantage or to a therapeutic disadvantage. Tumour cell types that have low DRF values, growing in an anatomical site where the dose-limiting normal tissues have a high degree of sparing, will be better treated by low dose-rate therapy. But the converse could also be the case. Radioresistant tumours that are well spared by low dose rate could be at an advantage if the limiting normal tissues (such as haemopoietic
tissues) have a poor capacity to recover. These conclusions apply equally well to hyperfractionation, for the use of many small dose fractions is radio-
biologically equivalent to a low dose rate.

The implication is that in the use of hyperfrac-
tionation or low dose-rate therapy far more atten-
tion needs to be given to selection of those tumour
types that will respond best, either by identifying
categories of tumour that have minimal low dose-
rate sparing or by developing predictive assays ap-
propriate to the individual patient.

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