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Tissue repair capacity and repair kinetics deduced from multifractionated or continuous irradiation regimens with incomplete repair

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Summary A model is proposed to account for cell survival after multiple doses, when the interfraction interval is insufficient for complete Elkind repair. In the limit of ever-increasing numbers of ever-smaller fractional doses, the model transforms into the accumulation model (Roesch, 1978) of survival after continuous irradiation. When it is adapted to describe tissue responses to isoeffective multifractionated regimens, wherein repair is incomplete, a generalization of the usually linear plot of reciprocal total dose *versus* dose per fraction is obtained, in which downward curvature is evident.

There is some advantage in studying tissue responses to multifractionated regimens with incomplete repair in the interfraction intervals, or continuous exposures at various dose rates since in addition to determination of *repair capacity* (defined by β/α) there is an estimate of *repair kinetics* (defined by the halftime $T_{\frac{1}{2}}$ for repair of sublethal injury). There is a saving in overall treatment time with either method, thereby reducing the influence of regeneration on the interpretation of the results.

The results of analyses of previously published data are presented to illustrate the use of the models. Estimated from the response of three acutely responding normal tissues in the mouse (jejunum, colon and bone marrow), repair halftimes ranged from 0.3–0.9 h and values of β/α were approximately 0.1 Gy^{-1}. From the response of mouse lung (LD50 for pneumonitis) to multifractionated regimens with incomplete repair, the repair halftime was estimated at 1.5 h and β/α was 0.27 Gy^{-1}. In the rat spinal cord β/α was 0.7 Gy^{-1} and $T_{\frac{1}{2}}$ was 1.5 h. Thus early and late-responding normal tissues may differ in both their repair capacity and repair kinetics, with the clinical implication that hyperfractionation to spare late effects preferentially will be limited by the slower rate of repair in these tissues.

The response of the gastrointestinal mucosa to doses of ionizing radiation, assayed by the microcolony technique, has been characterized after multifractionated irradiation of the jejunum (Withers *et al.*, 1975) and the colon (Withers & Mason, 1974). The number of fractions was limited by two factors: first, that the overall time was short enough that proliferation was negligible, and second, that the time between doses was long enough that intracellular repair processes were complete.

The purpose of this report is to describe the results of experiments in which the second of these restrictions, i.e. complete repair, was relaxed. A mathematical model of response to multiple doses with incomplete repair between doses is used to analyze the results, some of which have been published before (Withers 1975; Withers *et al.*, 1975; Withers & Mason 1974; Vegesna *et al.*, 1982; Ang *et al.*, 1983). The internal consistency of the method is checked by comparing the results obtained with different interfraction intervals, and its extension to continuous exposures is described.

Materials and methods

Experimental

The details concerning the experimental animals, the irradiation techniques, and the endpoints used have been published elsewhere (Withers & Mason 1974; Withers 1975; Withers *et al.*, 1975; Vegesna *et al.*, 1982; Ang *et al.*, 1983). A brief summary of the tissues studied and the endpoints used is presented in Table I.

Table I Repair capacity and repair kinetics:

Tissues, endpoints and nature of radiation exposure
1. Jejunum and Colon (microcolony assay, multifractionated with variable Δt).
2. Lung (LD50 (pneumonitis), multifractionated with $\Delta t = 3$ h).
3. Bone Marrow (LD50 (WBI), continuous with variable dose rate).

Data analysis

The models were developed from a generalization of the concept of "dose-equivalent of incomplete repair" (Oliver 1964), illustrated in Figure 1a. When a third dose is given, unrepaired injury from the second is equivalent to the dose θx, and from the first to the dose $\theta^2 x$ (Figure 1b). Therefore, the initial dose range $\theta x + \theta^2 x$ is lost when determining the response to the third dose. When generalized to n doses of size x, with interfraction interval Δt, the result is

$$\ln(\text{s.f}) = -n(\alpha x + \beta x^2) - \beta n x^2 h_n(\theta) \quad (1)$$

where $\theta = e^{-\mu \Delta t}$ and

$$h_n(\theta) = (2/n)\theta(1-\theta)^{-1}[n-(1-\theta^n)/(1-\theta)] \quad (2)$$

For tissue effect corresponding to target-cell effect $E = -\ln(\text{s.f.})$,

$$1/D_n = (\alpha/E) + (\beta/E)x[1 + h_n(\theta)] \quad (3)$$

in which $D_n = nx$. If total dose D and overall time T are held constant, while letting x and Δt tend to zero and $n \to \infty$, we obtain the accumulation model (Roesch 1978) of survival after continuous exposure at dose rate v:

$$\ln(\text{s.f.}) = -\alpha(vT) - \beta(vT)^2 g(\mu T) \quad (4)$$

or for tissue isoeffect:

$$1/vT = (\alpha/E) + (\beta/E)(vT)g(\mu T) \quad (5)$$

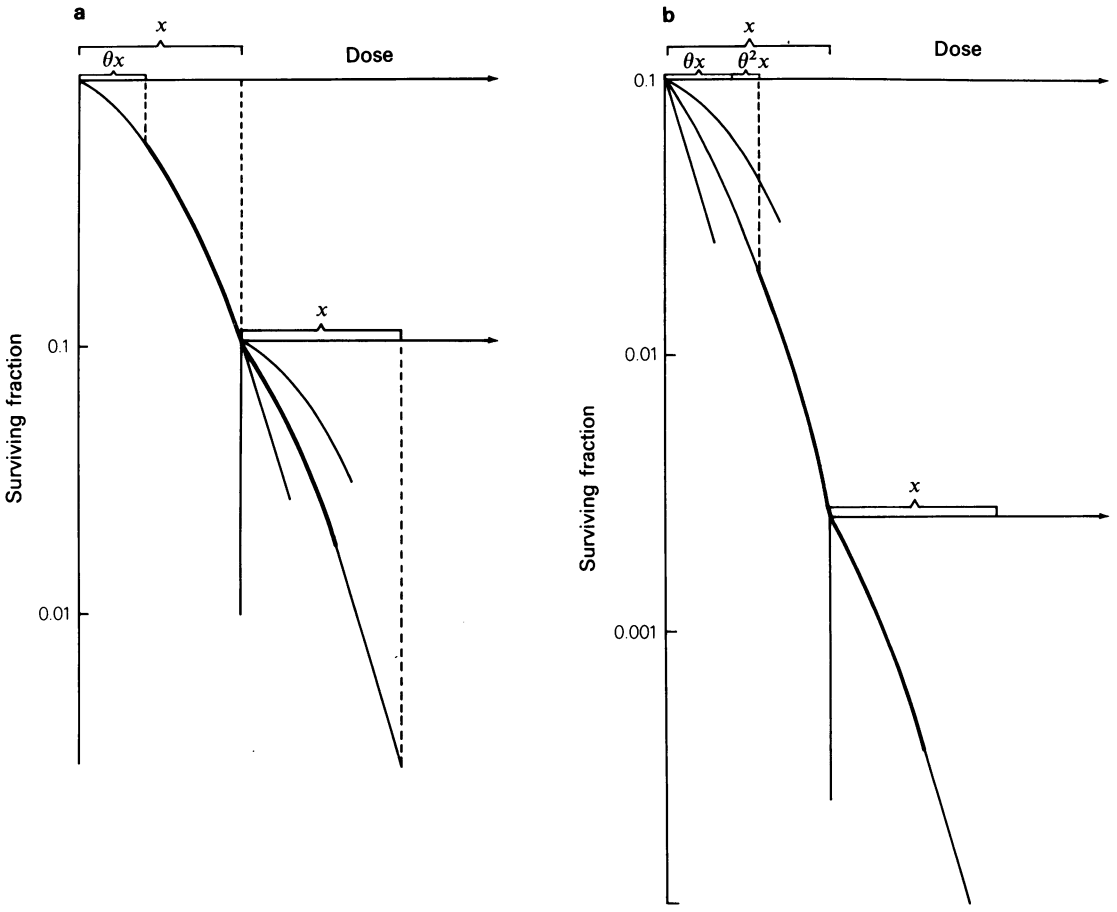

Figure 1 Dose-equivalent of incomplete repair. (a) The heavy portion of the dose-survival curve is repeated after a second dose of size x; the initial segment $\theta x = xe^{-\mu \Delta t}$ is not repeated. As Δt increases, an increasingly large part of the initial segment is repeated, until for large Δt the entire segment is recovered. (b) Dose-equivalent of incomplete repair, after 2 doses. The upper-left is transcribed from **Figure 1a.** After a third dose of size x, the part of the initial segment (heavy curve) is repeated that does not include θx (unrepaired from second dose) + $\theta^2 x$ (unrepaired from first dose).

The data were fitted using non-linear regression to the models Eqs. (1)–(5) as appropriate. Details of the calculations will be published elsewhere.

Results and discussion

Jejunum and colon

The progressive increase in sparing of jejunal mucosa with increasing interfraction interval is illustrated in Figure 2, which shows the responses to 5 doses separated by intervals of 0.5, 1 and 6 h. It is clear from the displacement of the curves that repair is incomplete after 1 h, or that significant regeneration had occurred in the $\Delta t = 6$ h schedule.

The dependence of sparing on length of interfraction interval was studied in a variety of fractionation schedules (Table II). Data were fitted using the model Eq. (1). Since single-dose determinations were made in conjunction with multifraction experiments with interfraction intervals of 1, 1.5, and 3 h for the jejunum, and 1 and 3 h for the colon (Table I), a consistency check of the model was suggested: the estimates of α, β, and $T_{\frac{1}{2}}$ obtained from experiments using the different intervals should be roughly equal. On the other hand, if there is a systematic trend in the parameter estimates with changing length of fractionation interval, doubt would be cast on the validity of the model.

Table II Fractionation regimens for jejunum and colon

	Δt (h)	*Number of fractions*
Jejunum	$\frac{1}{2}$	5, 10
	1	1, 2, 3, 5, 10, 15
	$1\frac{1}{2}$	1, 3, 10, 15
	3	1, 2, 3, 4, 5, 10, 15
	6	5, 10
Colon	1	1, 2, 3, 5, 10
	3	1, 2, 3, 5, 6, 8, 10, 15, 20

The results of this comparison are shown in Table III. There is little difference between the estimated survivial parameters and repair halftimes from the different intervals used in experiments on jejunum. The confidence interval (95%) for $T_{\frac{1}{2}}$ is large for the 3 h data, indicating that better quality estimates of repair kinetics may be obtained when a short Δt is chosen, in fact one too short for complete repair. This is especially so for the parameters obtained for colon, since confidence intervals are very wide for β and $T_{\frac{1}{2}}$ in the fit to the 3 h data.

With some assurance of the internal consistency of the model, the data were pooled and estimates of the parameters were obtained simultaneously from all the data (Table III). The goodness of fit is illustrated for some of the data in Figure 3. Two comments are important in connection with the fitting. First, ln(s.f.) is usually unknown with microcolony assays (amounting to an unknown constant, the log number of clonogens per circumference). In the present case, however,

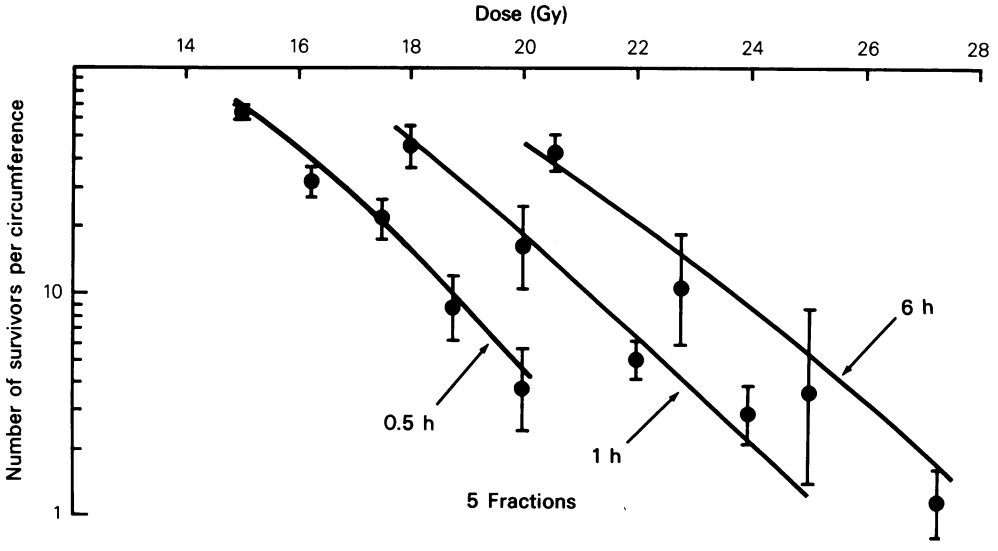

Figure 2 Increased sparing of jejunal mucosa after 5 fractions of gamma rays separated by increasing interfraction intervals.

Table III Consistency of estimates of survival parameters and repair halftime

	(colon)		
	$\Delta t(h)$		
	1	*3*	*All data*
#Animals	472	161	633
$\alpha(Gy^{-1})$	0.15 (0.13, 0.17)	0.15 (0.13, 0.17)	0.14 (0.13, 0.15)
$\beta(Gy^{-2})$	0.017 (0.016, 0.018)	0.15 $(-10^7, +10^7)$	0.019 (0.018, 0.020)
$T_{\frac{1}{2}}$(h)	0.96 (0.85, 1.07)	0.63 $(-10^7, +10^7)$	0.97 (0.91, 1.03

	(*Jejunum*)			
	$\Delta t\,(h)$			
	1	*1½*	*3*	*All data*
#Animals	245	289	363	897
$\alpha(Gy^{-1})$	0.20 (0.18, 0.21)	0.22 (0.21, 0.23)	0.20 (0.19, 0.21)	0.21 (0.20, 0.22)
$\beta(Gy^{-1})$	0.024 (0.023, 0.025)	0.024 (0.23, 0.025)	0.024 (0.023. 0.025)	0.024 (0.023, 0.025)
$T_{\frac{1}{2}}$(h)	0.47 (0.43, 0.53)	0.47 (0.38, 0.61)	0.44 (0.25, 2.2)	0.49 (0.46, 0.51)

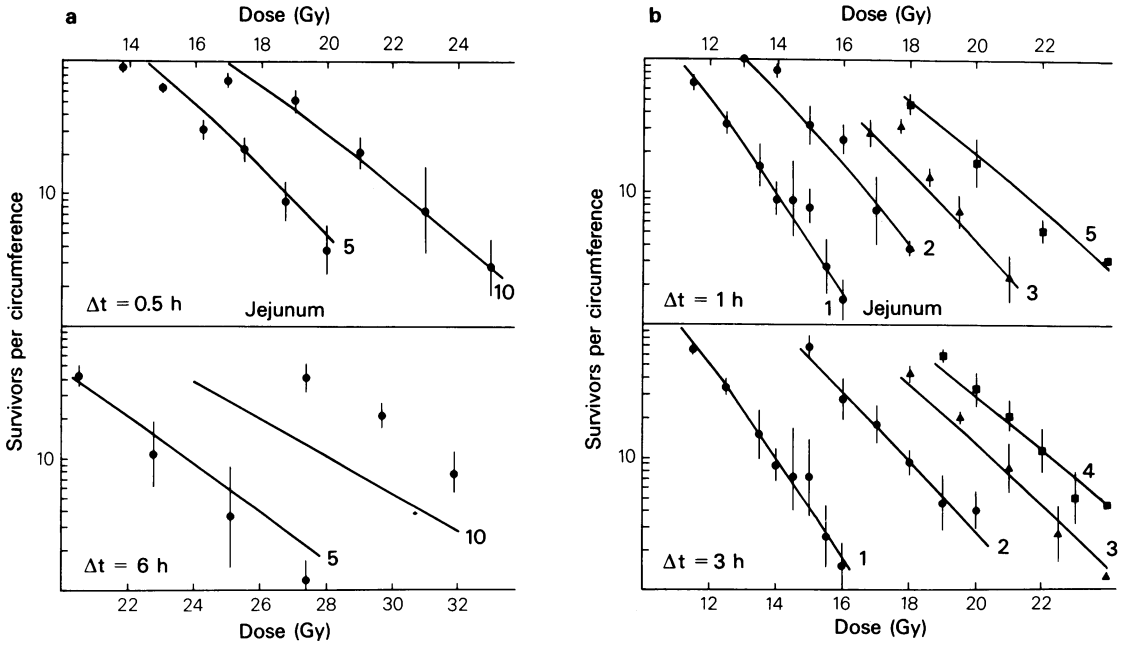

Figure 3 Fit of incomplete-repair model (Eq. (1) in text; data of Withers (1975 and unpublished)). (a) Response of jejunal mucosa to 5 or 10 fractions of gamma rays, with intervals 0.5 (upper) and 6 (lower) h. The solid curves show the fit to the pooled data (**Table II**). The influence of proliferation is evident for 10 doses separated by 6 h. (b) Goodness of fit to observed response of jejunal mucosa to fractionated regimens with intervals of 1 (upper) and 3 (lower) h. Excluded from the fit were data that showed evidence of proliferation (**Figure 3a** lower) or effect inversion (not shown).

estimates were available from previous studies of the jejunum (Thames *et al.*, 1981) and colon (Tucker *et al.*, 1983). In the fitting it was discovered that this constant may not be free, to be determined from least squares estimation. Instead, it was preferable to fix its value at a predetermined level. In practical terms, this means that proper application of these methods to data of the type shown in Figure 3 requires that a preliminary determination has been made of the log number of clonogens, as described by Thames and Withers (1980) or using other techniques.

Second, when large sets of data are pooled, as summarized by Table II, certain inconsistencies manifest themselves. There are, for example, cases where cell-cycle redistribution or parasynchrony might have influenced the results, as when the response to 20 fractions lay beneath the response to 15 fractions. As illustrated in Figure 3a, the fit to the combined data (solid curve) can lie well below the observations when treatment times were long, indicating that proliferation might have occurred. Data were suppressed from the fit (Figure 3b) when inversion of response occurred, or when it seemed possible (because of a consistent displacement of data from protracted experiments) that results had been affected by proliferation.

It is interesting to conjecture that the divergence shown in Figure 3a between predicted and observed survival when treatment times were long might offer the basis for quantification of regenerative response in these tissues, given a set of fractionation regimens for which it is certain that no proliferation occurs.

Lung

The LD50 for pneumonitis was determined in mice between 80 and 120 days after exposure to multifractionated doses of X-rays (Vegesna *et al.*, 1982), with 3 h interfractions intervals. As shown in Figure 4, there is downward curvature in the graph of reciprocal LD50 *versus* dose per fraction, instead of the straight line that should result under ideal conditions (Douglas & Fowler 1976). The solid curve shows the fit of the model Eq. (3) of incomplete repair, with estimates $\beta/\alpha = 0.27\,\mathrm{Gy}^{-1}$ (repair capacity) and $T_{\frac{1}{2}} = 1.5\,\mathrm{h}$ (repair kinetics), under the assumption that the curvature in Figure 4 is due to incomplete repair. The dashed line indicates what would have been expected if repair had been complete in the interfraction intervals (h = 0 in Eq.(3)).

Factors other than incomplete repair could be responsible for the observed result. For example, it could be argued that the downward displacement of the data in Figure 4 at small dose fractions occurred as a result of target-cell proliferation. If

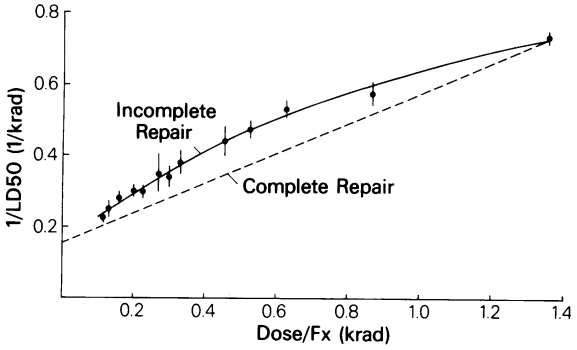

Figure 4 Downward curvature in graph of 1/LD50 (pneumonitis) *versus* dose per fraction (data of Vegesna *et al.*, 1982). Solid curve: fit of the model Eq. (3) of incomplete repair during 3 h fractionation intervals. Dashed line: predicted values with complete repair.

regeneration were exponential, with rate constant λ, and began after a negligible time lag, the appropriate model is

$$1/LD50 = (\alpha/E) + (\beta/E)x - (\lambda/E)\Delta t/x. \qquad (6)$$

The fit of the data to Eq. (6) is illustrated by the solid curve in Figure 5; the dashed line shows the predicted reciprocal LD50-dose per fraction line, had proliferation not occurred ($\lambda = 0$ in Eq.(6)). However, proliferation is an unlikely explanation of the downward curvature, for two reasons. First, the dose recovered in the most protracted regimens (7 days), as measured by the difference between the left-most data and the dashed line in Figure 5, is large (13 Gy) so that the dose recovered per day

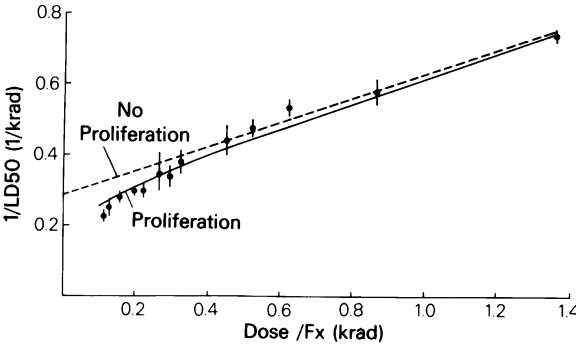

Figure 5 Downward curvature as explained by proliferation. Solid curve: fit of the proliferation model (Eq. (6) in text). Dashed line: predicted values in the absence of proliferation, assuming equal effect per fraction and complete repair.

would be in excess of that observed even in an acutely responding tissue such as mouse skin. Second, the estimate of λ, taking into account the extremes of possible values for E, leads to estimates of the doubling time of the proliferating population that range from less than 1 to slightly over 2 days. The slow turnover times of the most likely target cells, type II pneumocytes (Simnett & Heppleston 1966) and vascular endothelial cells of the lung (Evans *et al.*, 1969), are at variance with both these implications of Figure 5.

Slow repair (Field *et al.*, 1976) might also be offered as an explanation of the downward curvature. The dose recoverable from slow repair at 7 days, as measured in split-dose experiments, ranges from 1 Gy (Travis & Down 1981) to 3.5 Gy (Field *et al.*, 1976). While the possibility that slow repair was a factor in the most protracted regimens cannot be excluded, these doses are too small to account for the 13 Gy difference evident in Figure 5.

Bone marrow (continuous irradiation)

The dependence of LD50 for the bone-marrow syndrome on dose rate has been determined at 30 days in mice and the results will be published in full elsewhere. The fit of the tissue-effects version of the accumulation model, Eq. (5), is illustrated by the solid curve in Figure 6. The estimates of repair capacity and repair kinetics in mouse bone marrow were, respectively, $\beta/\alpha = 0.11\,Gy^{-1}$ and $T_{\frac{1}{2}} = 0.3$ h.

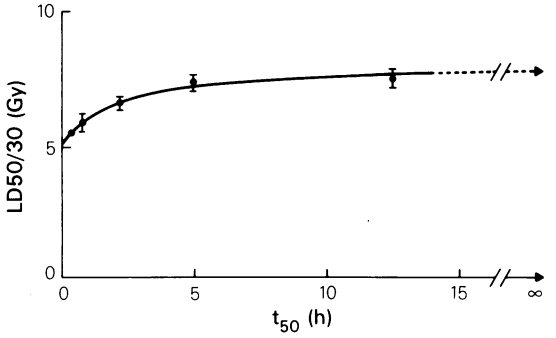

Figure 6 LD50 for bone-marrow death *versus* dose rate. Solid curve: fit of the accumulation model (Eq. (5) in text).

Conclusions

Two comments of a general nature may be made to summarize these findings. First, from the point of view of experimental design, it would appear that some advantage can be derived from studying tissue responses in terms of either multifractionated regimens with incomplete repair in the interfraction intervals, or continuous exposures at varied dose rate. Either technique affords estimates of both repair capacity and repair kinetics in a tissue, as opposed to traditional fractionation regimens, from which only repair capacity may be determined. In addition, there is a saving in overall time with either method, thereby reducing the influence of regeneration on the results.

Second, an interesting possible difference in repair kinetics between acutely and late-responding normal tissues emerges. While it has been noted previously that repair capacity (as measured by the ratio β/α) seems greater in the late-responding tissues (Thames *et al.*, 1982), it appears that repair kinetics is also different in these two groups, being slower in the late-responding tissues. The results are set out in Table IV, where the recently published studies of Ang *et al.* (1983) of the response of rat spinal cord to 2- and 4-fraction regimens with incomplete repair have been included for comparison.

If generally applicable, the results shown in Table IV would have important clinical implications for hyperfractionated radiotherapy: The potential for a therapeutic advantage derived from increased total doses given in the same time as conventional, but with markedly reduced dose fractions given several times per day (Thames *et al.*, 1983), would be limited by the slower rate of repair in the late-responding tissues.

Table IV Repair capacity and repair kinetics in early and late-responding normal tissues

Tissue	*Reference*	$\beta/\alpha\,(Gy^{-1})$	$T_{\frac{1}{2}}\,(h)$
Bone marrow	To be published	0.12	0.30
Jejunum	Withers (1975) and unpublished data	0.09	0.45
Colon		0.12	0.97
Lung	Vegesna *et al.* (1982)	0.27	1.5
Spinal cord	Ang *et al.* (1983)	0.71	1.4

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