Studies of the dose-effect relation

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Summary. Dose-effect relations and, specifically, cell survival curves are surveyed with emphasis on the interplay of the random factors – biological variability, stochastic reaction of the cell, and the statistics of energy deposition – that co-determine their shape. The global parameters mean inactivation dose, $\bar{D}$, and coefficient of variance, V, represent this interplay better than conventional parameters. Mechanisms such as lesion interaction, misrepair, repair overload, or repair depletion have been invoked to explain sigmoid dose dependencies, but these notions are partly synonymous and are largely undistinguishable on the basis of observed dose dependencies. All dose dependencies reflect, to varying degree, the microdosimetric fluctuations of energy deposition, and these have certain implications, e.g. the linearity of the dose dependence at small doses, that apply regardless of unresolved molecular mechanisms of cellular radiation action.

Key words. Cell survival; dose dependence; relative biological effectiveness; linear energy transfer; microdosimetry parameters of the dose dependence; repair; misrepair.

1 Parameters of the dose-effect relation

The topic of dose-effect relations encompasses nearly all aspects of radiation biology and it is addressed in most of the contributions to this review. The following treatment focuses primarily on the mathematical representation of cell-survival curves, but some of the considerations apply equally to other dose-effect relations and to the general problem of models and mechanisms. For a survey of experimental data the reader is referred to a subsequent chapter (Alper and Cramp[1]) and to the monograph of Elkind and Whitmore[11].

1.1 Random factors in the survival curve

When cells are exposed to cytotoxic agents, one obtains dose dependencies that deviate appreciably from a step function, and this departure from the simple threshold dependence can reflect biological variability, i.e., differences in the sensitivity of the exposed cells. The derivative, s(D), of the normalized survival probability, S(D), is then the differential distribution of the resistance:

$$s(D) = -\frac{dS(D)}{dD}, \quad (S(O) = 1) \tag{1}$$

S(D): fraction of cells that retain proliferative capacity after dose D

s(D) dD: fraction of cells inactivated by the additional dose increment dD.

Synchronization reduces one important source of variations: the changing sensitivity throughout the cell cycle. But even a synchronized cell culture exhibits marked deviations from the simple threshold reaction, and this reflects, at least partly, an inherently stochastic reaction of the cell. This 'indeterminism' may have its root in quantum mechanical uncertainties or in the practical impossibility of determining all critical parameters at any time. It is exemplified by DNA replication with its low but finite error rate, or – on a more 'macroscopic' level of intracellular processes – by the random distribution of the chromosomal material of a parent cell to its daughters.

The probabilistic response of the cell has, by itself, motivated few mathematical descriptions or formal models because it is too complex, and too poorly understood. Quantitative radiobiology, however, had from its inception the ambitious aim of a quantitative description[8,10,31,46], and this necessitated a consideration of all stochastic factors in the dose-response relation[22–24].

When phages, viruses, and certain bacteria are exposed to x-rays, the active fraction decreases exponentially with dose which reflects the randomness of energy transfer, i.e., of *hits* which were presumed to be clusters of ionizations or single ionizations[8,10]. The essential feature of the exponential relation is the inactivation of equal fractions of the surviving cells by equal dose increments:

$$S(D) = \exp(-\alpha D), \text{ hence: } \frac{dS(D)}{S(D)\,dD} = \frac{d\ln S(D)}{dD} = -\alpha \tag{2}$$

Here, α is the slope of the resulting straight line in the familiar semilogarithmic representation; it equals the frequency of inactivating events (lethal lesions) per unit dose. The constant value of α implies that the inactivation of the cell is not a cumulative process, but a result of single random events of energy absorption.

The ideas of target theory were substantiated in various studies of inactivation of enzymes or viruses, i.e., of macromolecular systems without repair[39]. This motivated attempts to explain sigmoid survival curves for higher cells in terms of accumulated acts of energy deposition. The resulting models account crudely for the statistics of energy absorption, but disregard other random factors.

They need to be compared to alternative models, and particularly to those that account for repair and misrepair. The comparison will be facilitated by general concepts which are considered next.

1.2 Mean and coefficient of variance

S(D) is the probability of a cell to retain its proliferative ability. It can be seen as probability distribution of the inactivation dose. The probability density is s(D).

The notion of the inactivation dose as a random variable is not entirely trivial, since one cannot determine the value of the variable for an individual cell. However, this does not lessen the applicability and utility of the concept. The most fundamental parameters of a probability distribution are its moments, and in particular the two first moments, in the form of the mean $\bar{D}$; and the variance, σ^2, of the inactivation dose[23, 32, 33]:

$$\bar{D} = \int D\, s(D)\, dD = \int S(D)\, dD$$
$$\sigma^2 = \overline{D^2} - \bar{D}^2 = 2\int D\, S(D)\, dD - \bar{D}^2 \qquad (3)$$

A related dimensionless parameter is the *coefficient of variance*. It measures the deviation of a dose-effect relation from a step function:

$$V = \sigma^2/\bar{D}^2 \qquad (4)$$

For an exponential relation V equals unity; for sigmoid survival curves it has smaller values. Figure 1 exemplifies this for a mammalian cell line in different phases of its cycle.

If two survival curves differ by a dose modifying factor, the mean inactivation doses, $\bar{D}$, will exhibit this ratio, but the values of V will be equal. The parameters $\bar{D}$ and V are of especial value, when survival curves for different cell lines need to be compared and when they exhibit differences that are not commensurable in terms of conventional parameters[37].

The variance σ^2, represents the joint influence of all random factors that co-determine the survival curve, including biological variability, stochastic reaction of the cell, and randomness of energy transfer. A synchronized population, for example in S phase where the shoulder tends to be largest (see fig. 1), can therefore have an appreciably smaller coefficient of variance, V, than an unsynchronized population. Similar considerations apply to densely ionizing radiations; as seen in figure 2, one obtains not only enhanced effectiveness, i.e., smaller values of $\bar{D}$, but due to the larger fluctuations of energy deposition also larger values of V.

In spite of the marked dependence on radiation quality it would be wrong to model a sigmoid survival curve merely in terms of the statistics of energy deposition in certain assumed target structures. A substantial part of the observed variance of the inactivation dose can reflect statistical factors other than energy deposition, and this must be taken into account in any quantitative treatment.

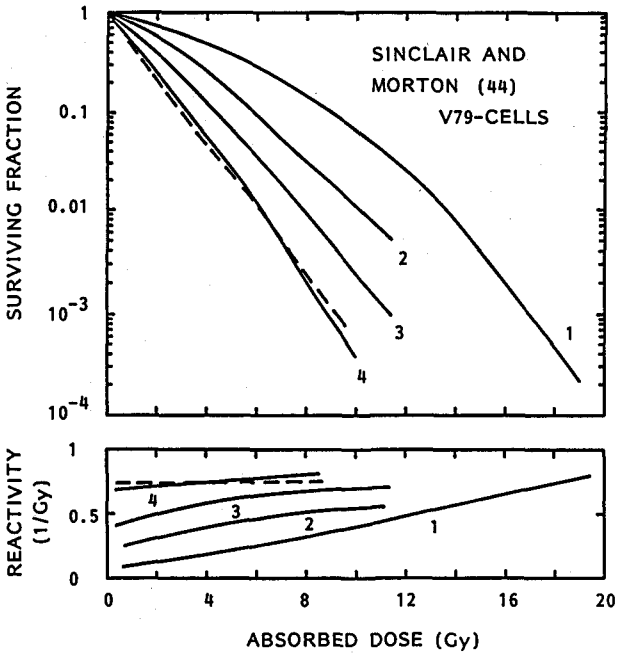

Figure 1. Survival of V-79 Chinese hamster cells exposed to x-rays in different phases of the cell cycle[34].

The dependencies, S(D), in the upper panel are those derived by Sinclair and Morton. Data points cannot be inserted because the dependencies incorporate substantial corrections for cell multiplicity. The corresponding reactivities, i.e. slopes (see subsection 1.3), are plotted in the lower panel. The assessment of error ranges would be difficult, but it is plausible that linear dependences $\alpha(D)$ (see Eq (6)) are also consistent with the data (see figs 3 and 4).

1: Late S-phase,	$\bar{D}$ = 4.40 Gy,	V = 0.52
2: Early S-phase,	$\bar{D}$ = 2.96 Gy,	V = 0.59
3: G1-phase,	$\bar{D}$ = 2.09 Gy,	V = 0.73
4: G2-phase,	$\bar{D}$ = 1.52 Gy,	V = 0.83
(broken lines: Mitosis,	$\bar{D}$ = 1.32 Gy,	V = 1)

1.3 The reactivity and the conventional parameters

Mean and variance are global parameters that represent the cellular reaction over the entire dose range. However, in considerations on mechanisms one is often interested in a restricted range of doses and therefore uses conventional parameters[11, 25]. The two principal ones are the reciprocals, D_1 and D_0, of the initial and the final slope of the (semi-logarithmic) survival curve. It is evident that D_0 need not exist, and that its presumed value can be considerably uncertain. Two further, related parameters are the values where the assumed asymptotic tangent intersects the ordinate and the abscissa. They are termed extrapolation number, n, and shoulder width, D_q, and are subject to the same restrictions, but are frequently quoted, since they are readily, if loosely, estimated by visual inspection of the survival curve.

Figure 3 demonstrates, in terms of the survival curve for V-79 cells in late S-phase, that two response functions may seem to be equal with regard to the conventional parameters, while they are, in fact, substantially different. The difference is brought out by the parameters $\bar{D}$ and V, and it is accentuated in figure 4 by a linear representation which is instructive although it is, by mere

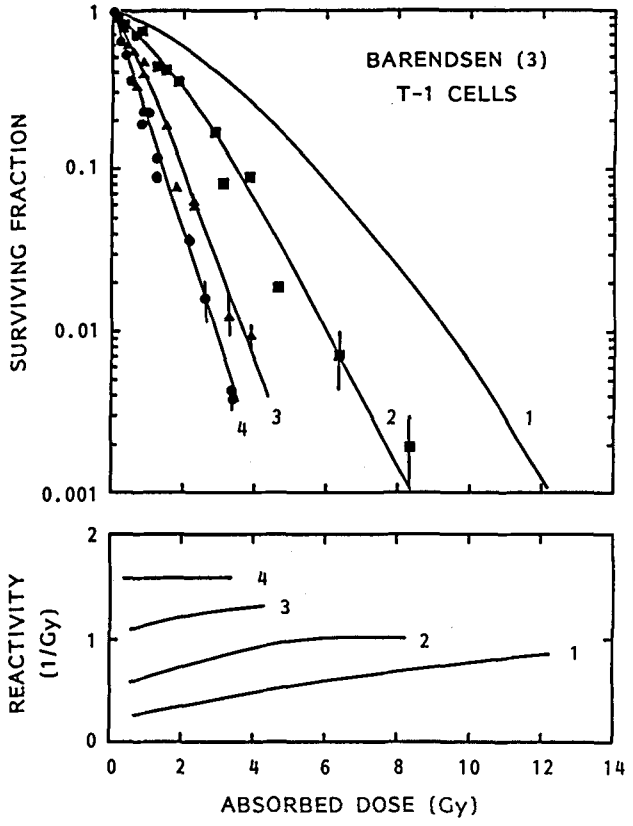

Figure 2. Survival curves for the human kidney cell line T-1 exposed to x-rays and to helium ions of varying LET (2).
The dependencies, S (D), in the upper panel are those given by Barendsen. The corresponding values of the reactivity are given in the lower panel. Linear dependencies $\alpha(D)$ (see Eq (6)) would also be consistent with the data.

1: x-rays (250 kVp),	$\bar{D} = 2.78$ Gy,	V = 0.54
2: LET = 25 keV/μm,	$\bar{D} = 1.63$ Gy,	V = 0.69
3: LET = 61 keV/μm,	$\bar{D} = 1.00$ Gy,	V = 0.76
4: LET = 85 keV/μm,	$\bar{D} = 0.64$ Gy,	V = 1

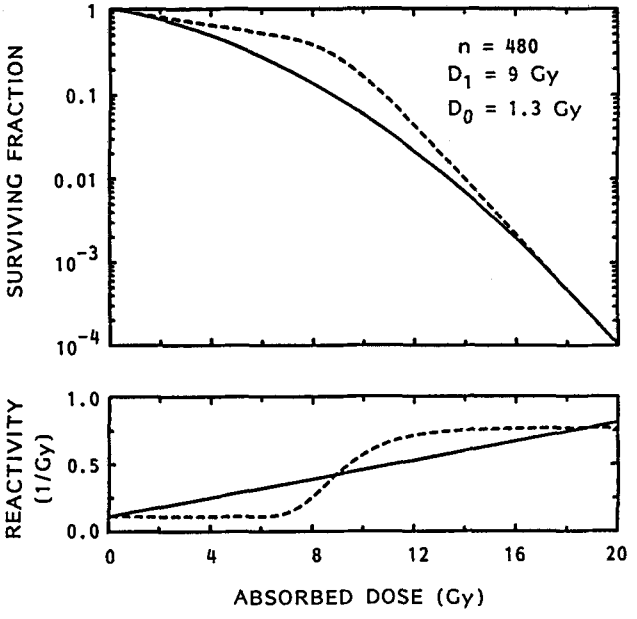

Figure 3. The dependence for late S-phase from Fig. 1 approximated by a linear-quadratic relation (solid lines, Eq (6), a = 0.11/Gy, b = 0.017/Gy^2; $\bar{D}$ = 4.47 Gy, V = 0.50).
The parameters D_0 and n do not exist, but values inferred from the terminal part of the curve would equal those of the broken curve.
The multi-target equation (broken lines, modified Eq (9), α = 0.11/Gy, b = 0.66/Gy, n = 480; $\bar{D}$ = 6.08 Gy, V = 0.38) has the same apparent conventional parameters, but differs substantially and is not consistent with the data.

convention, entirely uncommon in cell-irradiation studies.

The slope in the semi-logarithmic diagram is the central notion in models that seek to explain the sigmoid survival curve in terms of a dose dependent reduction of repair efficiency. Because of its importance the quantity is plotted in this article together with all survival curves. Hug[22,23] has introduced the term 'reactivity', which is here used with the notation $\alpha(D)$:

$$\alpha(D) = -\frac{d \ln S(D)}{dD} \qquad (5)$$

As stated earlier, $\alpha(D)$ may be considered as the rate of inactivating lesions per unit increment of dose. But this need not be taken too literally, since loss of proliferative ability could include a gradual loss of stability that enhances spontaneous failure rates by a multiplicity of defects. Such gradual change is indicated by altered kariograms even in surviving irradiated cells.

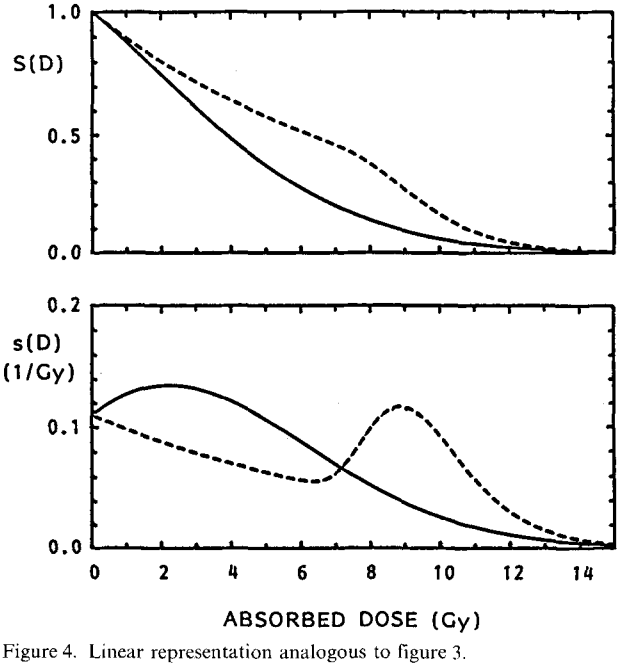

Figure 4. Linear representation analogous to figure 3.

The 'hockey-stick' curve in figure 3 with its step-like increase of the reactivity can be readily distinguished from the observed trend of the data. But one would require fairly accurate experimental data to distinguish the linear increase of the reactivity which corresponds to the linear-quadratic survival curve

$$\ln S(D) = -aD - b D^2 \quad \text{hence: } \alpha(D) = a + 2bD, \quad (6)$$

and the more general relation which has been postulated (see Haynes [17], Hug and Kellerer [22, 23]) to account for an exponential loss of compensation or repair ability with increasing dose:

$$\alpha(D) = a + 2\frac{b}{c}(1 - \exp(-cD)),$$
$$\ln S(D) = -\left(a + \frac{2b}{c}\right)D + \frac{2b}{c^2}(1 - \exp(-cD)) \quad (7)$$

The coefficients are here written in such a form that Eq (7) agrees with Eq (6) for $c = 0$. Eq (7) replaces the unlimited increase of $\alpha(D)$ by convergence towards an asymptotic value. The resulting expression is somewhat awkward, but the concept is simple and can be readily appreciated from the example in figure 5.

It is understandable that an equilibrium value of $\alpha(D)$ should be reached under the usual condition where a dose dependence is determined with constant dose rate, i.e. with exposure times proportional to dose. The set of curves in figure 5 is, indeed, representative of survival curves obtained with different constant dose rates [4, 16]. It

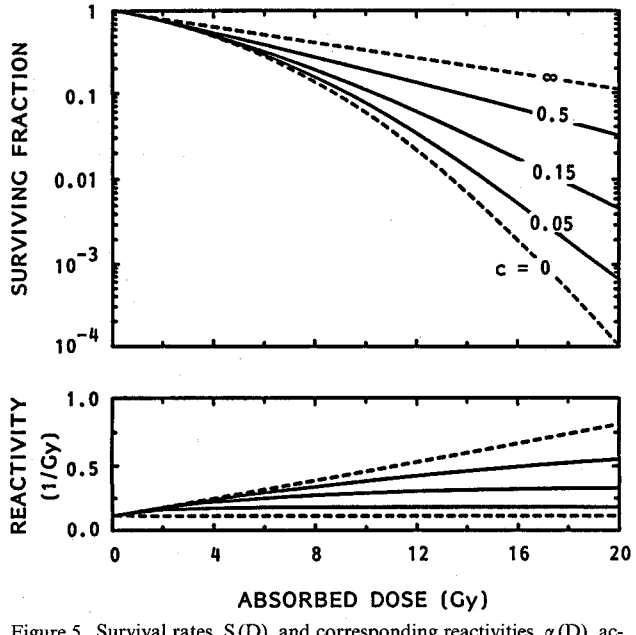

Figure 5. Survival rates, S (D), and corresponding reactivities, $\alpha(D)$, according to Eqs (6) and (7) with $a = 0.11/Gy$ and $b = 0.035/Gy^2$.
The parameters c are noted in the diagram; $c = 0$ corresponds to Eq (6), i.e. the linear-quadratic relation.
The set of curves is consistent with survival curves obtained at different constant dose rates.

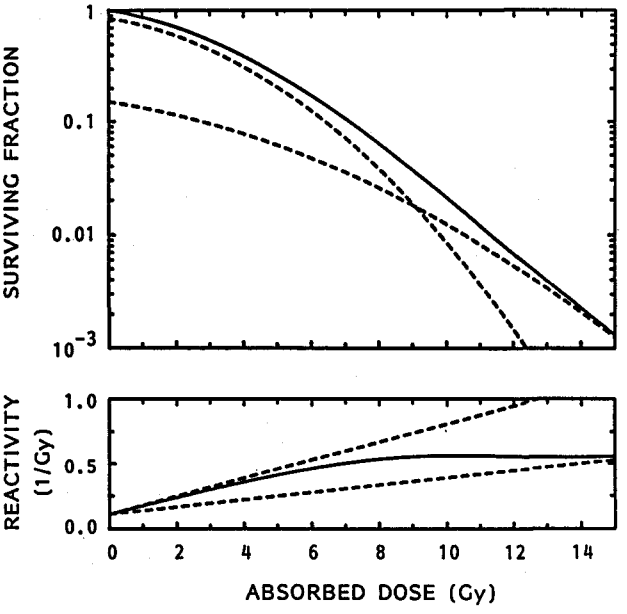

Figure 6. A superposition of two linear-quadratic relations (broken lines) which results in a seemingly exponential tail, i.e. a plateau of the reactivity, of the dose dependence for the mixed population (solid lines).

is less certain whether Eq (7) – rather than the simple linear-quadratic dependence – needs to be invoked for instantaneous exposures or dose dependencies obtained with fixed exposure time (see also Kellerer and Rossi [34]). Survival curves often appear to have an exponential tail, but this could be an artefact. In experiments one deals with imperfectly synchronized or otherwise different cells and the resulting superposition is correctly judged in a linear plot of the survival curve. The diagram of ln S (D), however, can be misleading because it distorts the superposition. The overall reactivity, i.e. slope, is the average of the reactivities only at low doses; at high doses it can be substantially smaller than the average for the subpopulations and it tends then towards the minimum reactivity within the entire population; figure 6 illustrates the situation in terms of a simple example of only two subpopulations. Asymptotic values of $\alpha(D)$ must, accordingly, be judged with caution; they may represent a minor, comparatively insensitive subpopulation of cells. Disregard of this unavoidable bias may generate the perhaps mistaken conclusion that survival curves of mammalian cells are always exponential at high doses.

2 Models and equations

Even without an inquiry into models one can catalogue certain main ideas. The resulting incompleteness may be more apparent then real, since equivalent postulates are frequently expressed in terminologies and equations that differ widely in form but not in substance.

A multiplicity of models are concerned with the sigmoid survival curve for sparsely ionizing radiations, the oxy-

gen enhancement ratio (OER), the disappearance of the non-linear component at reduced dose rates (time factor), and the enhanced relative biological effectiveness (RBE) of densely ionizing radiations. Only the survival curve will be addressed here, but there are implications for related problems. All models have in common the notion of damage accumulation that causes the non-linear dose dependencies. The accumulation could be one of lesions that need to reach a critical level, but such a threshold assumption has little factual support. It is more commonly assumed that lesions interact pairwise[6, 9, 13, 15, 30, 34, 47, 48], and the multitude of mechanisms which have been envisaged have in common the fact that the interaction results when the spatial proximity of lesions interferes with regular repair and causes misrepair. Alternatively, lesions could interfere indirectly; they might be less rapidly repaired when their abundance exceeds the capacity of repair, and this may result in a higher rate of fixation[14, 38]. A further concept refers not to the direct or indirect interaction of lesions, but explains the shoulder of the dose effect relation in terms of radiation damage to a repair capacity[1, 17, 22, 44].

2.1 Target-theory equations

The multi-hit and the multi-target equations[10, 46] represent the general notion that random acts of energy deposition accumulate lesions up to a critical threshold. Assuming identical and statistically independent hit events in presumed identical targets one can utilize the Poisson formula for the probability of i events at an expected frequency bD. Postulating a critical threshold of N events one obtains the multi-hit equation:

$$S(D) = \sum_{i=0}^{N-1} p(i) = \sum_{i=0}^{N-1} \exp(-bD) \cdot (bD)^i/i! \qquad (8)$$

Another target-theory equation has found wider application for the representation of survival curves because of its greater simplicity and the fact that it exhibits finite values n or D_q (see subsection 1.3).

The multi-target equation corresponds to the postulate of n presumed equal targets that need to be eliminated by individual random events for loss of viability of the cell. The resulting survival relation is:

$$S(D) = 1 - (1 - \exp(-bD))^n \qquad (9)$$

where b is the frequency of events per target per unit dose. Both Eqs (8) and (9) have initial slope $\alpha(0) = 0$ and asymptotic slope b. Only Eq (9) has a finite extrapolation number, n.

To account for the generally observed initial slope of survival curves, it is customary to include in Eq (9) the additional exponential factor $\exp(-\alpha D)$. The resulting equation with 3 parameters can fit many survival curves; but the simpler linear-quadratic relation (or its extension Eq (7)) is usually more suitable (see, for example, figs 3 and 4).

The target-theory equations are special cases of a general scheme of linear Markov processes[23, 32], and the more general treatment has led to statements that can replace certain conjectures of target theory. For example, it has been shown that the coefficient of variance, V, can never be less than the inverse of the postulated number, N, of random events in a model. Equality applies only in the case of Eq (8). The hit number, 1/V, associated with a dose dependence is thus merely a lower bound for the number of random steps which may, in reality, be far larger.

Equations (8) or (9) have, inspite of their familiarity, little importance, since the associated models are too crude. The randomness of energy deposition is merely one factor in a sigmoid survival curve, and even this factor is inadequately described in the target-theory models. The most striking feature in microdosimetry[27, 29, 40, 41] is the highly variable magnitude of energy depositions by charged particles in microscopic and submicroscopic sites, and no model of cellular radiation effects can be valid that disregards these variations.

2.2 Lesion interaction, misrepair, and reduced repair efficiency

Lesions may be produced at a rate k per unit dose, and may be repaired with probability p. One obtains, then, proportionality between the number of lesions and absorbed dose and an exponential survival relation:

$$\ln S(D) = -aD, \quad \text{with } a = k(1 - p) \qquad (10)$$

However, some repair mechanisms will tend to fail when lesions occur in close proximity, for example, if excision repair extends over a region with two opposing strand breaks to cause a double strand break, or if two chromosome breaks occur in sufficient proximity to induce misrepair and consequent aberrations. The probability of regular repair may then decline exponentially with the concentration of lesions and, therefore, with dose. The resulting relation is identical with Eq (7) in section 1.3 (see fig. 5):

$$\alpha(D) = k(1 - p(D)) = k(1 - p \cdot \exp(-cD)) \qquad (11)$$

For small values of c one obtains the linear-quadratic relation:

$$\alpha(D) = a + bD, \text{ with } a = k(1 - p) \text{ and } b = \frac{ckp}{2} \qquad (12)$$

One can call this, with equal justification, a lesion-interaction or a misrepair model.

Other possibilities have been considered and may, in view of experimental observations, be equally likely. With increasing concentration of lesions there could be an overload of repair capacity with decreasing chance of a lesion to be repaired before fixation. A suitable quantitative description could again be Eq (11), and this means that

the different possible mechanisms cannot be inferred or rejected on the basis of dose-effect relations alone.

A further but largely equivalent notion is that of depletion of a repair system, tentatively termed O repair [1, 45]. For this process, too, one could assume an exponential decrease of repair efficiency with dose and, again, one would expect dependencies such as Eqs (11) or (12).

Actual dependencies would, of course, have to relate also to microdosimetric parameters which determine, for different radiations, the spatial proximity of lesions. If repair overload were to apply, one would have to examine the spatial constraints of this process, and similarly one would have to ask whether an assumed depletion of a repair system is localized or disperse. At present these models are still too tentative to permit a quantitative microdosimetric formulation which could render them verifiable or falsifiable.

One observes generally, and this is an essential conclusion, that survival curves alone do not permit discrimination between the various modes of lesions interaction, repair overload, or damage to the repair systems. To support such discrimination by fitting survival curves would be formalism; answers need to be provided by experiments specifically designed to identify molecular mechanisms.

3 Microdosimetric considerations

Random energy absorption in the cell has long been the central topic of radiation biology. The historical development led from the merely heuristic target-theory models to Lea's analyses in terms of linear energy transfer and track structure [36], and to the concepts and methods of microdosimetry conceived by Rossi [27, 29, 40, 41]. These developments and their attempted use towards the identification of DNA lesions and their repair, misrepair, and fixation is beyond the scope of this survey. But some essentials will be noted.

3.1 Event frequencies and the linear dependence at low doses

The principal tenet of microdosimetry is – in spite of its name – that dose cannot and need not be measured in microscopic regions. Cellular effects are determined by actual energy concentration, i.e. by the specific energy [26, 27], and this can differ substantially from the absorbed dose which is merely a statistical mean value. To understand the role of microdosimetry one needs to consider the general magnitude of the fluctuations of specific energy.

If a mammalian cell is exposed to an absorbed dose of 1 Gy of x-rays, its nucleus (assumed to be of 5 μm diameter) is traversed by roughly 2000 energetic electrons. On the level of the entire nucleus one can then disregard the fluctuations of energy absorption. When the same cell is exposed to the much more effective dose of 1 Gy of energetic neutrons, its nucleus may be traversed by only 5 or 10 densely ionizing recoil protons and the specific energy can then differ substantially from the absorbed dose.

The knowledge of event frequencies permits simple conclusions of great generality and of considerable importance to radiation protection. If the cell nucleus experiences on the average only one event per 0.1 or 0.2 Gy, one must disregard the probability of multiple events at the substantially smaller doses that are of interest to radiation protection. The absorbed dose determines, therefore, merely the number of cells that experience one particle traversal. The energy deposition in one of these cells and the probability for resulting damage, such as loss of viability, chromosome aberrations, or mutations, is dependent merely on the type and energy of the ionizing particle and not on dose. It follows that cellular effects are proportional at low doses to the number of traversed cells and, therefore, to dose. The condition is evident for densely ionizing radiations, but it applies, at small doses of fractions of a mSv, with equal certainty also to sparsely ionizing radiations.

The conclusion has broad implications because it removes the possibility of a threshold for genetic damage produced by ionizing radiation. For radiation carcinogenesis it makes the absence of a threshold at least likely, because somatic mutations are the initiating steps that may, although with small probability, lead to a tumor. Non-linearities could arise if this small probability of a transformed cell to break through control and defence mechanisms were also dependent on dose; but this may be unlikely at small doses. With essentially the same arguments one concludes that there can be no dependence on dose rate at small doses where one deals merely with single hits in individual cells. This, too, is of particular relevance to radiation protection where one cannot assume a continued decrease of effectiveness at decreasing dose rate.

Dose dependencies for cell survival, for mutations, chromosome aberrations, or cell transformations suggest that proportionality to dose and absence of a time factor apply even at substantially higher doses, of fractions of a Gray, of sparsely ionizing radiations, and the microdosimetric arguments that refer to much smaller doses may, therefore, appear somewhat academic.

There have, however, been striking observations that suggest unexpected complexities even at low doses. An adaptive response – an enhanced resistance to the induction of chromosome breaks – was seen after human lymphocytes were exposed to 5–100 mGy of sparsely ionizing radiation [50]. A substantial increase of transformation rates was seen with neutrons [19, 20, 21], but not with α-rays [18], when small doses were protracted over one to several hours. Such unresolved observations highlight the value of microdosimetric conclusions that remain unaffected by the complexity of cellular processes. The conclusions are essential, although they refer only to

very small doses of sparsely ionizing radiations. In radiation protection one is concerned with the possible effects even of minute doses to large populations, and linearity of the dose dependence and absence of an influence of dose rate are then important.

3.2 Threshold models in terms of microdosimetry

In analogy to target theory, threshold models have been formulated also in terms of microdosimetry. The simplest postulate is that of a critical threshold of specific energy in the entire nucleus of the cell. However, the high event frequency for sparsely ionizing radiation makes it clear that this would lead almost to step functions, i.e. to values V (see section 1.2) that are far less than those of observed survival curves or other dose dependencies. To preserve the notion of a threshold, a smaller sensitive site has been invoked, but this conflicts with the known distribution of the sensitive target, DNA, throughout the cell nucleus. Simple threshold models in terms of microdosimetry must therefore be rejected.

A modified approach[5] takes account of the stochastic reaction of the cell and attempts to unfold the fluctuations of 'cell dose', i.e. specific energy in the nucleus, from the observed dose dependence to obtain a response function in terms of specific energy. However, the same objections apply: For the entire nucleus the fluctuations are irrelevant, at least for sparsely ionizing radiations, and the assumption of a smaller reference region conflicts with the known distribution of DNA.

A less ambitious approach can lead further. Studies with soft x-rays, i.e., short-ranged electrons[14,48], and investigations with spatially correlated ions[42,43] have shown that lesions are formed with enhanced frequency by increased energy concentrations on the nanometer scale. Disregarding the unresolved problem of the sigmoid dependence at higher doses one can then examine initial slopes of the dose dependencies obtained with different types of ionizing radiations. For a multiplicity of cylindrical target structures – rough approximations of DNA – simulation studies were then utilized to determine assumed energy thresholds that would be reached with probabilities by the different radiations that parallel the observed data[14]. The uncertain target geometry and the unlikely threshold assumption may make the results hypothetical, but the hypotheses can motivate, and have motivated, further studies.

3.3 The second order process and the linear-quadratic relation

The multiplicity of parameters in the possible models makes it desirable to ask for the simplest common denominator, and this may be the treatment of a second order process in radiation action.

Essential concepts, but even important details, were formulated by Lea[36]. He assumed that pairs of lesions, for example chromosome breaks, could interact to damage the cell. The yield of lesions would then be proportional to the square of the concentration of lesions. Radiation, unlike chemical toxins, produces highly unhomogeneous microscopic concentrations of energy, of free radicals, and of resulting lesions. Even when the dose is reduced, the local concentrations remain high around the tracks of individual charged particles, and this leads to a linear intra-track component in the dose relation which is proportional to ionization density and is superimposed on the quadratic inter-track component. The resulting linear-quadratic dose dependence is the most common representation of dose-effect relations.

Microdosimetric data have simplified the argumentation without changing its essentials[34,35]. The ratio of the linear to the quadratic coefficient has the dimension of a specific energy and it is determined by the mean energy produced by a single charged particle within a region over which damage can interact. These concentrations can be far higher for densely ionizing than for sparsely ionizing radiations, and this explains the high relative biological effectiveness of densely ionizing radiations at small doses.

Rossi has emphasized that the cellular lesions may be the result of a second order process, i.e. of dual action, but that manifest effects on the cell or tissue level need not exhibit the linear-quadratic dose dependence. However, the added complexities cancel, when the effects of two types of radiations are compared, and this has been supported by various studies of the increase of the RBE of neutrons with decreasing dose[34,35]. This type of analysis has been applied to such diverse effects as chromosome aberrations, radiation cataracts, or radiation induced neoplasms.

The implicit use of the linear-quadratic dose dependence is equally important in the determination of iso-effect doses in fractionated radiotherapy where the linear-quadratic dose dependence has been variously employed[3,12,49]; the familiar Ellis formula can be extended[28] in its range of validity by this relation. General parameters of the survival curve, as treated in section 1.2, have been utilized for the estimation of organ dependent parameters that are required in this context[37].

The identification of specific molecular mechanisms has had less tangible results. From the sigmoid dose dependencies for cell survival, but also for chromosome aberrations, damage accumulation over the order of one micrometer has been inferred. The studies with soft x-rays and with correlated heavy ions have demonstrated substantially enhanced interaction probabilities in the nanometer range, and this has been combined with the assumption of interdependence over longer range, but with greatly reduced probabilities, to explain the shoulder of the dose dependence. The tool for this type of analysis[35,51,52] is the *proximity function*[31] which measures spatial correlations of energy transfers in charged particle tracks. The function is utilized to compute the yields of a second order process with distance dependent reaction probabilities, and it defines the linear compo-

nent in the linear-quadratic dependence as a distance-averaged measure of energy concentrations, due to individual charged particles.

The general treatment of a second order process is more flexible than earlier microdosimetric formulations, but it is also less falsifiable because it admits of a variety of interpretations, including lesion interaction by misrepair, repair overload, or damage to a repair system. Added studies will be required to investigate the parameters that influence – and might influence differently [7] – the linear and the quadratic component of the dose relation, and can thus help to identify the nature of the interdependence of energy transfers on the nanometer and on the micrometer scale. The treatment is, in fact, a necessary tool rather than a model.

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The role of repair in radiobiology

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Summary. Apart from cancer and mutation induction, radiobiological effects on mammals are mostly attributable to cell 'death', defined as loss of proliferative capacity. Survival curves relate retention of that capacity to radiation dose, and often manifest a quasi-threshold ('shoulder').
The shoulder is attributable to an initial mechanism of repair ('Q-repair') which is gradually depleted as dose increases. Another form of repair, which is not depleted ('P-repair'), increases the dose required to deliver an average of one lethal event per cell (dose 'D_0'). Neither form of repair can unambiguously be linked with repair of defects in isolated DNA. An important initial lesion may well be disruption of the complex structural relationship between the DNA, nuclear membrane and associated proteins. One form of P-repair may be restoration of that structural relationship.
Key words. Repair; cell survival; fractionation; sublethal; potentially lethal; relative biological effectiveness (RBE); DNA breaks; DNA synthesis; P-repair; Q-repair.

Introduction

Before nuclear energy became available for both peaceful uses and weaponry, interest in the effects of ionizing radiation on man was centred on its use for medical diagnosis and for the treatment of disease – mainly cancer. Attention had to be paid also to hazards of accidental or unavoidable incidental exposure: for example, to radiologists and radiographers. Some radiobiologists and radiotherapists recognized that cancerous tumours regressed after irradiation because the malignant cells had lost their capacity to reproduce themselves; but concurrent damage to the normal tissues of treated patients was not in those days regarded as the consequence of randomly occurring events in individual cells. Studies on intracellular effects in organized tissues focussed on the induction of chromosomal aberrations (plant cells, tissue cultures), and on the induction of heritable changes in germ cells, mainly in plants and the fruit-fly, *Drosophila*. After 1945, however, considerable effort was made in many countries to learn in general about radiation effects on mammals, for the most part laboratory animals, with the object of extrapolating to man. A good deal of attention was initially devoted to 'LD 50' doses, i.e. those necessary to kill 50% of an animal population within a certain time. A voluminous literature accumulated on biochemical and physiological effects of irradiation. Because death was thought to be attributable to complex changes in metabolism, consequent on irradiation. But an experiment by Quastler[33], conceived in very simple terms, demonstrated that there were basically three modes of death, depending on the dose range. The lowest range, 4–6 grays for most mammalian species, killed 50% of the animals within 15–30 days. Death occurred at 4–5 days after irradiation by doses in the range 10–100 grays ; after greater exposures, death occurred within a few hours.
It gradually emerged that the first two of these three modes of death depend on the loss of proliferative capacity by stem cells in tissues that require continual replenishment of functioning cells. This is the case with haemopoietic' tissue and with the cells populating the surface of the villi in the intestinal tract. One of the most easily evident effects of radiation on cells of all classes is indeed