Annals of Radiation Therapy and Oncology

Research Article
Published: 24 Nov, 2017

Biophysical Models, Microdosimetry and the Linear Quadratic Survival Relation

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Abstract

The Linear Quadratic (LQ) survival relation is presented along with its derivation from the Theory of Dual Radiation Action (TDRA) and the related Microdosimetric Kinetic (MK) model. The role of the LQ model in the reporting and interpretation of experimental and clinical studies of the effects of ionizing radiation exposure is discussed. The definition of a Biological Effective Dose ($BED_{\alpha/\beta}$) based on the LQ model is given and its use to compare fractionated radiation treatment courses is described. The implications of heterogeneity of radio sensitivity among the cells of an irradiated cell population with regard to the LQ survival relation and the calculation of the $BED_{\alpha/\beta}$ are described. The results of important early studies reported using survival constants defined by the target model are reviewed and related to the survival constants of the LQ survival relation. The dependence of the relative biological effectiveness (RBE) on the linear energy transfer (LET) is presented.

Introduction

Since the discovery by Puck and Marcus in 1956 [1] of a method to produce individual countable colonies of mammalian cells, each derived from the growth of a single cell, it has been possible to measure the capacity of ionizing radiation to destroy the colony forming ability of mammalian cells, referred to as cell reproductive death. This is important because radiation induced cell reproductive death is the beneficial effect sought in the treatment of cancer and other neoplastic disease. It is also the cause of the morbid effects of the often unavoidable exposure of normal tissues and organs to radiation. Another important observable effect of exposure to ionizing radiation is the induction of chromosome aberrations, the study of which is central to the investigation of radiation genetics, carcinogenesis and risk assessment. Furthermore, formation of a lethal chromosome aberration is the dominant pathway by which radiation causes mammalian cell reproductive death.

The Linear Quadratic (LQ) relation between dose and the fraction of cells that survive able to form a colony after exposure to ionizing radiation (Equations 4 below) is used to report and interpret the results of radiobiological experiments. It also provides the basis for comparison of the clinical effect of the treatment of a variety of cancers with radiation of various Linear Energy Transfer (LET) quality administered using a variety of continuous and fractionated treatment schedules. Models from which the linear quadratic relation is derived and its application in experimental radiobiology and clinical radiation oncology are the subject addressed here.

The models described here were formulated so as to apply to irradiation of a homogeneous population of cells, all of which have the same sensitivity to radiation. Cell populations studied in the laboratory and treated in clinical radiation oncology are almost always a heterogeneous mixture of cells with diverse radiation sensitivities. The implication of heterogeneity of cell radio sensitivity is discussed in the appendix and at places in the text.

Multi Target Single Hit Survival Model

The multi-target single hit model of radiation induced cell killing preceded the linear quadratic model as the favored way to quantitatively express the result of mammalian cell radiation survival experiments. It is described here to compare and contrast it with the LQ model and to relate the results of important early cell survival experiments to the parameters of the LQ model.

Figure 1 shows the surviving fraction of HeLa cells measured by Puck and Marcus [1]. The attention of radiobiologists was drawn to the exponential survival that develops as dose increases toward infinity and the suggestion of zero slope in the limit of zero dose. These same features were also found in subsequent experiments with other cultured mammalian cells. They are compatible with a model in which it is postulated that a cell has n structures (targets) each of which may be

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Received Date: *23 Aug 2017*
Accepted Date: *14 Nov 2017*
Published Date: *24 Nov 2017*

Citation:
Hawkins RB. Biophysical Models, Microdosimetry and the Linear Quadratic Survival Relation. Ann Radiat Ther Oncol. 2017; 1(2): 1013.

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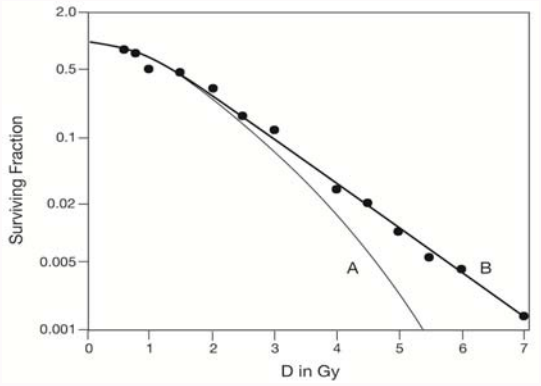

Figure 1: Fraction of cell culture of HeLa cells that survive exposure to x-rays from Puck and Marcus [1]. Curve A is a linear quadratic curve that fits the experimental points from zero to about 3 Gy with α=0.25 Gy^{-1} and β=0.19 Gy^{-2} as determined from line A of Figure 2. Curve B is fit by inspection to the experimental points over the whole range of dose. The limiting high dose exponential line of curve B corresponds to D_0=1.2 Gy and D_0^{-1}=0.83 Gy^{-1}. Its extrapolated intercept at zero dose corresponds to n=2 of the target model (equation 2).

inactivated by passage of a radiation induced high energy charged particle through or near it. Cell reproductive death results when all n targets are inactivated. This leads to the survival relation [1-3],

$$S = 1-\left(1-e^{-k_2 D}\right)^n \qquad (1)$$

in which S is the fraction of cells that retain the ability to form a colony after exposure to an instantaneously administered dose of D Gy. The value of n is the number of targets per cell that must be inactivated to reproductively kill the cell and k_2 is the constant that governs the exponential survival of each target. Later it became apparent that experimental cell survival relations may have a non zero slope at zero dose. This was accommodated by a modification that added a single hit exponential mechanism. This is,

$$\begin{aligned} S &= e^{-k_1 D}\left[1-\left(1-e^{-k_2 D}\right)^n\right] \\ &= e^{-k_1 D}\left[1-\left(1-ne^{-k_2 D}+\frac{n(n-1)}{2!}e^{-2k_2 D}-.....+e^{-nk_2 D}\right)\right] \\ &\approx ne^{-(k_1+k_2)D} \end{aligned} \qquad (2)$$

The second line of equation 2 contains the binomial expansion. As dose increases so that $k_2 D$ becomes greater than about 2 the approximation of the third line is approached and the relation of ln S to dose, becomes a simple exponential,

$$\begin{aligned} \ln S &= -(k_1+k_2)D+\ln n \\ &= -D/D_0+\ln n \qquad D_0=(k_1+k_2)^{-1} \end{aligned} \qquad (3)$$

Based on this model, experimental survival curves of mammalian cells were characterized by two parameters, D_0 and n. The value of D_0 is the increment of dose that produces a survival fraction of $1/e$ for the exponential relation approached with increasing dose. The value of ln n is the intercept of the exponential relation for high dose extrapolated back to zero doses. The value of n is the target number. It serves as a measure of the shoulder of the survival curve.

Linear Quadratic Model

By about 1980, the linear quadratic (LQ) cell survival relation became favored over that of the target model by many radiobiologists and radiation oncologists [4]. For a population of cells, all of which have the same radio sensitivity, expressed in the constants α and β, the linear quadratic relation for the fraction that survive able to form a colony after instantaneous exposure to dose D is given by the LQ model as,

$$S = e^{-\alpha D-\beta D^2} \text{ or } -\ln S = \alpha D+\beta D^2 \qquad (4)$$

The values of α and β are characteristic of the cell type and conditions of irradiation. The exponential form of the equation 4 survival relation is consistent with cell reproductive death being due to radiation induced formation of discreet lethal lesions distributed individually at random among the cells of the irradiated population so as to conform to a Poisson distribution with average number of lethal lesions per cell ‹w› given by,

$$\langle w\rangle = \alpha D+\beta D^2 \text{ or } \frac{\langle w\rangle}{D}=\frac{-\ln S}{D}=\alpha+\beta D \qquad (5)$$

The second of equations 5 displays the linear increase in the average number of lethal lesions formed per unit dose as dose increases that is an important property of the LQ model. When cell survival is represented in a plot of -lnS/D *vs.* dose D the linear quadratic survival relation becomes a line with intercept at zero dose equal to α and slope equal to β. Linear quadratic survival, or deviation from it, is easy to recognized by inspection when survival is plotted this way.

As illustrated in Figure 1, experimentally determined mammalian cell survival after exposure to X- or Gamma irradiation from about 0 Gy to 3 Gy conforms at least as well to the LQ survival relation represented by equations 4 and 5 as to the relation of equations 2 and 3. As dose increases to greater than about 3 Gy the surviving fraction of most mammalian cell populations, plotted as -ln S *vs.* D, becomes the simple exponential described by the target model. The continuously curving, downwardly concave, linear quadratic survival relation (curve A of Figure 1) diverges from the experimental survival values and from the target model relation as dose increases.

The discrepancy between experimental survival and the linear quadratic model at higher dose is explained by heterogeneity of sensitivity to radiation among the cells of the irradiated population [5]. Consider that a targeted population of mammalian cells will generally consist of a heterogeneous mixture of subpopulations each of which is characterized by a different pair of values of α and β. Heterogeneous radio sensitivity may be present even among cells that are genetically homogeneous. For instance, the value of α and β of quiescent cells is generally not equal to that of actively replicating cells. Among those actively replicating, the values of α and β vary with age in the cell cycle. Additionally, in a malignant tumor there are zones of cells that have different access to the capillary circulation and, along with other differences, have different levels of oxygen tension.

From inspection of Figure 2 it can be appreciated that the survival of the heterogenous exponentially growing population of cultured He La cells obeys a linear quadratic relation, marked by line A, as dose decreases to approach zero. It obeys a different linear quadratic relation, marked by line B, as dose increases toward infinity. The slope of line B is the lowest value of beta among the cells of the population, called β_H. The value of β_H is indistinguishable from zero in many heterogeneous cell populations so that line B is close to horizontal as shown in Figure 2. As such it coincides with the high dose limiting

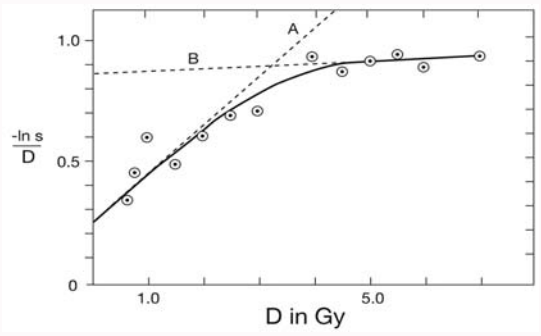

Figure 2: Surviving fraction of HeLa cells from Puck and Marcus [1] replotted from Figure 1 as –ln *S/D vs. D*. Line A is the linear quadratic relation that governs cell survival in the limit of low dose with α=*0.25* Gy^{-1} and β=*0.013* Gy^{-1}. Line B is the linear quadratic survival relation that governs it in the limit of high dose with α_H=*0.84* Gy^{-1} and β_H=*0.013* Gy^{-1}.

purely exponential relation expected from the target model.

Let α_H be the intercept of line B at zero dose. Note that both D_0^{-1} and α_H are the slope of the –ln*S vs.* dose relation in the limit of high dose so that D_0^{-1} effectively equals α_H.

It is difficult to measure cell survival at doses less than 0.5 Gy to 1 Gy. When able to be measured, the survival curve in this low dose range indicates some cells become more sensitive to radiation than expected from extrapolation of the LQ survival relation established by measurement at higher dose. This is referred to as low dose hypersensitivity and induced radio-resistance. It is the result of a low level of activity of the enzymatic processes that are involved in the repair of radiation induced DNA lesions. Instantaneous exposure to more than 0.5 Gy to 1.0 Gy or to a rate of continuous exposure greater than about 6 Gy to 10 Gy per hour is apparently necessary to fully induce the enzyme systems that protect against radiation injury to DNA in many cells [6,7]. Low dose hypersensitivity and induced radio-resistance is not addressed in the models discussed here. The survival relation for dose less than 1.0 Gy is taken to be that extrapolated from the LQ relation followed at higher dose.

The linear quadratic survival relation of equations 4 and 5 is favored over that of the target model because it is derivable from the theory of dual radiation action (TDRA) [8,9] and related models [10-14] that are based on the binary combination of radiation induced lesions to form a lethal lesion. As discussed below, these models are consistent with the experimental finding that production of lesions in DNA is linearly related to dose, that repair of radiation induced DNA lesions between exposures leads to increased survival and with the importance of chromosome aberrations in cell reproductive death. Finally, the relatively simple mathematical form of equations 4 and 5 encourages their use in laboratory and clinic.

A biologically effective dose ($BED_{\alpha/\beta}$) based on the LQ model was introduced by Barendsen [15] to compare the effect of courses of radiation treatment given in different increments of fractionated dose. The value of $BED_{\alpha/\beta}$ is defined as the dose required to produce the same surviving fraction as the course of interest if the radiation were given as an infinite number of infinitely small fractions of a reference low LET radiation over the same time span.

Let s be the fraction of cell that survive each of the n exposures to dose d that constitute a fractionated course of radiation treatment. Let α be the linear survival constant for the radiation used in the course of interest and α_R be that for a reference low LET radiation such as high energy X-rays or cobalt 60 gamma radiation. Let S the fraction of the cells that survive the course, is defined as the number of viable cells present immediately after the last exposure divided by the number present immediately prior to the first. The defining equation for $BED_{\alpha/\beta}$ is given by: $-\ln S=\alpha_R BED_{\alpha/\beta}$. For the linear quadratic model this becomes,

$$\begin{aligned} BED_{\alpha/\beta} &= \frac{-\ln S}{\alpha_R} = \frac{-n\ln s}{\alpha_R} - \frac{T\ln 2}{\alpha_R \tau_2} \\ &= \frac{n\left(\alpha d + \beta d^2\right)}{\alpha_R} - \frac{T\ln 2}{\alpha_R \tau_2} \\ &= RBE_1\left[nd\left(1+\frac{d}{\alpha/\beta}\right) - \frac{T\ln 2}{\alpha_R \tau_2}\right] \end{aligned} \quad (6)$$

In which $-\ln S=\alpha d+\beta d^2$ and $RBE_1=\alpha/\alpha_R$. The value of RBE_1 is the relative biological effectiveness of the radiation used in the course in the limit of zero doses (see equation 46). More generally, let the value of RBE_S be defined as the ratio d_R/d_H in which d_R is a dose of low LET reference radiation and d_H is a dose of a high LET radiation that produce the same surviving fraction equal to s. The value of T is the time between the first and last exposure and τ_2 is the time for the treated tumor population to double its volume in the absence of exposure to radiation. The time dependent term in equation 6 corrects the surviving fraction for exponential growth of the irradiated population during the time span of the radiation course. The value of τ_2 includes the effect of the average time from mitosis to mitosis of cycling cells, of there being a fraction of the cells that are quiescent and not cycling and cell loss from causes other than radiation exposure. Each fraction of dose d is given once or twice a day, in a few minutes or less so as to be effectively instantaneous. If more than one exposure is given in a day, they are separated by enough time for completion of the repair of the radiation induced lesions in DNA, which is usually 4 to 6 hours. The value of $BED_{\alpha/\beta}$ has units of Gy. Courses of treatment that have the same $BED_{\alpha/\beta}$ are regarded as equivalent in their effectiveness in destroying cancer or in causing injury to a normal tissue or organ.

Equation 6 as written refers to a population of cells with homogeneous linear quadratic radio sensitivity expressed in a common value of α and β. The effect of heterogeneous radio sensitivity on the important value of the α/β ratio as it appears in equation 6 is discussed in the appendix [5].

The value of τ_2 in the time dependent term does not allow for changes in the growth of the population that are induced by exposure to radiation. These may include radiation induced delay in the progress of cells through the cell cycle and acceleration of cell proliferation from decrease in the average cell cycle time and recruitment of quiescent cells into cycle stimulated by elimination of cells during the course and by chemical signals from tumor and host tissue [16]. The choice of volume doubling time in the correction for cell proliferation during a radiation course assumes these effects are a relatively minor perturbation of the proliferation of the cells of tumor or tissue. An alternative is to replace the volume doubling time in the absence of radiation exposure with the potential doubling time (τ_{pot}) as more representative of the rate of repopulation during the course. The value of τ_{pot} is the population doubling time if all cells are cycling and there is no cell loss. Its value is the average time from mitosis to mitosis of cycling cells divided by the fraction of cells that are cycling, called the growth fraction. A lag time is sometimes subtracted from T to account for delay in radiation induced acceleration of growth

referred to as accelerated repopulation [17].

The important ratio α/β characterizes the radiation response of the cells that make up various cancers, and the cells upon which the integrity and function of normal tissues or organs depends. The value of α/β for cells irradiated *in situ* in animal or patient can be estimated from the effect of variation of the dose per fraction on the total dose of a course of radiation treatment needed to produce a specific effect related to cell survival [15,18]. It is assumed that a given level of organ failure, for instance the onset of moist desquamation of skin or paralysis from spinal cord injury, develops when the surviving fraction of the cells upon which the effect depends is decreased to below a critical level. Similarly, it is assumed that a given probability of control or cure will be achieved when the surviving fraction of viable cells in an irradiated neoplasm is decreased to a critical level.

Let S^* be the critical surviving fraction level. Let D^* be the total accumulated dose to the organ needed to produce the specified injury or probability of tumor control when given as n exposures, each of dose d over time period T. The value of S^* is generally not known. However, it will be the same no matter what combination of fractional and total dose is needed to produce the specified organ injury or tumor control probability. Assume the linear quadratic survival relation of equation 4 governs each of the exposures of dose d. Then,

$$-\ln S^* = n\left(\alpha d + \beta d^2\right) - \frac{T \ln 2}{\tau_2}$$

$$= \alpha D^* + \beta D^* d - \frac{T \ln 2}{\tau_2} \quad \text{with } D^* = n \times d \tag{7}$$

Rearranging this gives a relation between $1/D^*$ and d.

$$\frac{1}{D^*} = \left(\frac{\tau_2}{-\tau_2 \ln S^* + T \ln 2}\right)\alpha + \left(\frac{\tau_2}{-\tau_2 \ln S^* + T \ln 2}\right)\beta d \tag{8}$$

The value of T will vary with the number of fractions in the course unless the time between fractions was adjusted to make T the same for courses with different dose per fraction and fraction number. This is not usually the case in experimental and clinical studies of the effect of changes in fractionation. Thus, the bracketed time dependent coefficient in equation 8 may depend on d. However, variation of T among the fractionated courses being compared is often small. Furthermore, if the value of $-\tau_2 \ln S^* >> T \ln 2$, the dependence of the bracketed coefficient on T will be suppressed. This is usually the case as the volume doubling time τ_2 is often significantly longer than T and S^* is likely to be less than 0.5. If the bracketed coefficient has no significant dependence on T, a plot of $1/D^*$ versus d will be linear for a homogeneous population of cells with linear quadratic survival. The slope and the intercept at d equal zero of the line defined by equation 8 can be determined. The ratio of the intercept to the slope equals the α/β ratio characteristic of the important cells in the tissue or cancer as they exist and function in the living animal or patient. A plot based on equation 8 is referred to as an iso-effect plot. A schematic drawing of an iso-effect plot for irradiation of a population with heterogeneous radiosensitivity is shown in Figure 4 and discussed in the Appendix.

Plots of equation 8 have been obtained from observation of the control of experimental tumors and the occurrence of various organ injuries produced by different schedules of fractionated irradiation of animals. Clinical experience with different fractionation schedules used for cancer treatment has been assembled to produce iso-effect

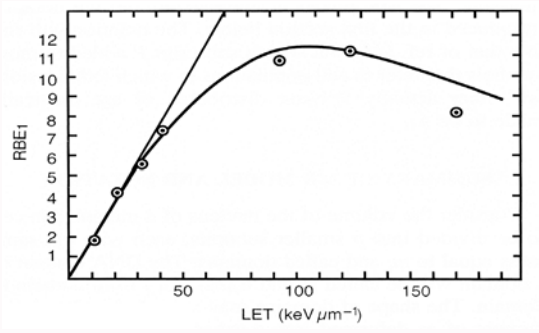

Figure 3: RBE_1 versus LET (L_D) for V79 cells synchronized in late S phase from experiment of Bird et al [14,33]. The reference low LET radiation is 250 kVp x-rays with $\alpha_R=0.064$ Gy^{-1} and $\beta_R=0.0165$ Gy^{-2}. The straight line is $RBE_{1P}=0.02+0.19L_D$. The concave downward curve is equation 43 with cross section $\sigma=18.2$ μm^2 corresponding to a circle with diameter of 4.8 μm.

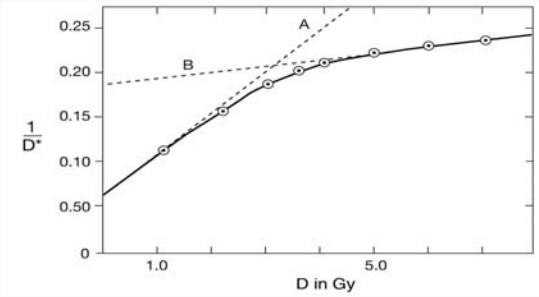

Figure 4: Schematic drawing of the isoeffect relation (equation 8) between the reciprocal of the total dose of a course of radiation treatment that produces a specified level of effect (D^*) when given in increments of dose equal to D Gy per fraction to a population of cells with heterogeneous radio sensitivity like that shown in Figures 1 and 2. The α/β ratio that applies to fractional dose in the low dose range is the ratio of the intercept to the slope of line A and that which applies to fractional dose in the high dose range is the ratio of the intercept to the slope of line B.

plots used to estimate the α/β ratio for the cells of human tissues or organs at risk in radiation treatment and for the cells of human cancers. Tables of the α/β ratio for various organs and tumors obtained this way are available for exposure to the low LET radiation of high energy gamma and X-rays [19-22]. There is consistency of α/β values obtained in animal and human studies. The effect of heterogeneous radio sensitivity on the estimation of the value of α/β from equation 8 is discussed in the appendix [5].

Radiation injury that develops during a course of radiation treatment, or in the few weeks following the end of a course, such as desquamation of skin, oral or pharyngeal mucositis and diarrhea from enteritis, is referred to as an early response. Tabulated values of the α/β ratio are in the range of 6 Gy to12 Gy for cells related to early responses [19-21]. However, the α/β ratio of a heterogenous population of cells is different in the low and high dose ranges defined by lines A and B of Figure 2 and 4 [5]. That obtained from the ratio of intercept to slope of line A is α/β as shown in equation A1 of the appendix. The alpha beta ratio in the high dose range from the ratio of intercept to slope of line B is α_H/β_H. With the rare exception of a negative value of β, α_H/β_H is greater than α/β. The tabulated values of the alpha beta ratio are generally based on iso effect studies that include fractional dose greater than 3 Gy to 5 Gy. This has the effect of increasing the estimate of the alpha beta ratio from that based on line A toward that of line B. Because of this, many of the tabulated values of α/β in the range of 6 Gy to 12 Gy are greater than the value

that is correct for the calculation of $BED_{\alpha/\beta}$ for a course with fractional dose less than 3 Gy to 5 Gy. This is illustrated for the early responding mouse jejunal crypt cells described below.

Radiation injury that develops several months to years after the end of a radiation course, for example, spinal cord injury, brain necrosis, kidney failure, telangiectasia, skin atrophy, subcutaneous fibrosis, bone and cartilage necrosis and respiratory failure from pulmonary fibrosis, is referred to as a late response. The tabulated values of the α/β ratio for cells related to late response tends to be in the range of 1 Gy to 6 Gy [19-21].

The tabulated value of α/β for the cells of tumors irradiated *in situ* in the living animal or patient ranges from 0.4 to 36 [19-22]. Absent a better estimate, a default value of α/β=10 Gy is often used for calculation $BED_{\alpha/\beta}$ for malignant tumors. Tumors with a high component of non cycling cells and slow cell replication, associated with a slow response to radiation treatment, tend to have the lowest α/β ratios. A notable example is prostate cancer [22] with α/β estimated as 1.1. With an appropriate assignment of the α/β ratio, $BED_{\alpha/\beta}$ can be a useful measure with which to compare treatment courses and select an optimal dose and fractionation schedule in clinical radiotherapy [5].

Relation of Target and Linear Quadratic Models and *In Situ* Cell Survival

Unlike the ratio α/β the value of α or β is not able to be determined from an iso effect study using equation 8 because of lack of a value for S^*. Experiments have been devised that allow the number of cells of an irradiated tissue that survive able to form a colony to be counted [23-30]. A linear dependence of the log of the number of surviving colony forming cells on dose was found signifying exponential survival as expected from the high dose limit of the target model. The value of the slope of the relation was interpreted as D_0^{-1} of the target model per equation 3 and reported as a value of D_0. The values of D_0^{-1} reported from these studies are effectively values of α_H for a heterogeneous population.

The heterogeneity in some malignant cell populations may be so great that the value of β becomes negative [5]. This is not found for the cell populations of normal tissues and mammalian cells in culture. If β is positive, α_H will be greater than α and $\alpha \leq D_0^{-1}$. The surviving fraction versus dose relation as dose approaches zero is not well defined in many of these experiments so that a reliable estimate of α is not available. The most useful survival parameter with which to characterize the radio sensitivity of the cell population of tissue or tumor is α.

With the expectation that $\alpha \leq D_0^{-1}$ some examples of the determination of the survival of colony forming stem cells of normal tissues exposed to radiation *in situ* and reported as a value of D_0 are as follows: For mouse stem cells that generate the circulating cells of blood [23], the value of D_0 is 0.95 Gy corresponding to $\alpha \leq 1.05$ Gy^{-1}. For the stem cells of mouse skin [24] D_0 is 1.35 Gy corresponding to $\alpha \leq 0.74$ Gy^{-1}. For cells that generate mouse sperm [25], D_0=1.8 Gy corresponding to $\alpha \leq 0.55$ Gy^{-1}. For the cells that generate the tubules of mouse kidney [26,27], D_0= 1.5 Gy corresponding to $\alpha \leq 0.67$ Gy^{-1}. For cells that generate rat thyroid, D_0 is 0.95 Gy corresponding to $\alpha \leq 0.51$ Gy^{-1} [28].

A complete survival curve was able to be obtained for mouse jejunal crypt cells and rat mammary cells [29,30]. Over the dose range from zero to about 5 Gy , the curve for jejunal crypt cells fits to the linear quadratic relation of equation 3 with values of $\alpha = 0.12$ Gy^{-1} and $\beta = 0.095$ Gy^{-2} and $\alpha/\beta = 1.26$ Gy [5]. A line tangent to the high dose region of the ln *S vs. D* curve has a slope equal to $\alpha_H = 0.43$ Gy^{-1} which simulates the value of D_0^{-1} as defined for the target model of equation 1. Thus, for the jejunal crypt cells the measured value of α from the low dose range is equal to about 28 percent of the simulated value of D_0^{-1}. The surviving fraction of the rat mammary cells fits to the target model relation with D_0^{-1}=0.77 Gy^{-1} and extrapolation number n=6 [30]. The experimental surviving fraction fits the linear quadratic relation in the dose range from zero to about 5 Gy with α=0.148 Gy^{-1}and β=0.049 Gy^{-2}and α/β ratio equal 3.02 Gy. Thus for rat mammary cells α in the low dose range is about 19 percent of the value of D_0^{-1}.

The values of D_0 reported for the cells of epithelial tissues and the finding of α equal to 19 and 28 percent of the value of D_0^{-1} in these two instances suggests that α of the cells that maintain the function and integrity of epithelial tissues and the depletion of which is responsible for the acute early developing radiation injury is in the range of about 0.10 Gy^{-1} to 0.20 Gy^{-1}. This is similar to the value of α found for several immortalized mammalian cell lines that resemble the cells of a cancer [1,31-36]. The survival of mouse stem cells that generate the circulating cells of blood is exponential with little if any shoulder evident to dose as low as one Gy. The value of α for these cells is close to that of D_0^{-1} and approximately 1.0 Gy^{-1}. In general, the greater the value of the ratio α/β of the low dose range linear quadratic survival relation, the less downward concavity of the ln *S vs.* dose survival curve, the more nearly equal are its the initial and final slope and the more nearly α equals D_0^{-1}.

Theory of Dual Radiation Action

The importance of the binary combination of radiation induced lesions in the formation of chromosome aberrations was appreciated even before the chemical structure of the chromosome was known [37-39]. The breakage and reunion, and exchange models of chromosome aberration formation were developed [40-42] based on binary combination of repairable radiation induced lesions. The Theory of Dual Radiation Action (TDRA) provides a generalization of the biological consequence of binary combination of radiation lesions, referred to as sublesions, to produce an effector lesion that in turn results in an observable cellular effect such as a chromosome aberration or cell reproductive death [8, 9]. The linear quadratic survival relation of equations 3 and 4 is consistent with formation of the lesion responsible for cell reproductive death from binary combination of radiation induced sub lesions as proposed in the TDRA [8]. The linear quadratic survival relation can be inferred from the TDRA as follows.

Let the relative biologic effectiveness of two radiations of different LET quality be called the *RBE* of the higher LET radiation and be given by,

$$RBE = D_L / D_H \qquad (9)$$

In which D_L is the dose of the lower LET radiation needed to produce a specified level of effect and D_H is the dose of a higher LET radiation needed to produce the same level of effect. The generalization implied by the TDRA was originally proposed by Kellerer and Rossi [8] on the basis of the observation that for a number of different radiobiologic effects, including cell reproductive death and dicentric chromosome aberrations, the *RBE* is proportional to $D_H^{-1/2}$, that is,

$$RBE = \left(\lambda / D_H\right)^{1/2} \qquad (10)$$

It is assumed that the observed radiation effects, particularly cell reproductive death, can result from a single radiation induced effector lesion in a cell. For cell reproductive death, an effector lesion is a lethal lesion. Let ε be the average number of lethal effector lesions per cell. The survival relation of cultured mammalian cells exposed to high LET irradiation, for instance high energy neutrons, is a simple exponential with linear decrease in survival on semi log plot for all or nearly all the accessible dose range. That is, for the neutron irradiation, equation 3 or 4 has an undetectably small value of β, at least in the lower dose range. It follows that production of the effector lethal lesions is proportional to D_H. That is,

$$\varepsilon = k_H D_H \qquad (11)$$

From equation 10 and 11 we have that $D_H=D_L^2/\lambda$ so the dependence of ε on D_L will be quadratic, that is, letting $k=k_H/\lambda$

$$\varepsilon = kD_L^2 \qquad (12)$$

This suggests that for low LET irradiation, effector lesions are created from the binary combination of sublesions, each of which is created from a different particle track through the nucleus. An effector lesion also is apparently created from a pair of sublesions arising from a single particle track produced by the higher LET radiation. Since survival of exposure to low LET radiation, for most cells, has a detectable decrease in the limit of zero dose, lethal lesions must also be created from combination of sublesions from a single particle track of low LET radiation, though to less degree than for high LET radiation. It was proposed from this that binary combination of sub lesions to produce a lethal lesion is the dominant way radiation causes cell reproductive death. Combination of sub lesions in the same track contributes more to the formation of lethal lesions for high than low LET radiation. Binary combination of sub lesions from different tracks contributes prominently to the formation of effector lesions from exposure to higher doses of low LET radiation. Combining these two ways of producing effector lesions to compute their total number gives,

$$\varepsilon = k\lambda D + kD^2 \qquad (13)$$

For cell reproductive death equation 13 becomes the linear quadratic relation of equations 3 and 4 in which α is identified with $k\lambda$ and β with k. The value of ε is the average number of lethal lesions per cell and equal to $\langle w \rangle$ of equation 4.

As the LET of radiation increases from that of high energy photon radiation to about 70 to 100 $keV \times \mu m^{-1}$, and possibly beyond, the value of α significantly increases but the value of β increases little, if at all [43-45]. This implies the dependence of cell survival on LET resides predominantly in λ, as does the *RBE* for cell reproductive death.

The particle tracks of higher LET radiation produce ionizations that are closer together than those produced by lower LET radiation. The greater tendency of higher LET radiation to produce binary combination of sub lesions that arise in the same particle track is an effect of the more closely spaced ionizations of higher LET tracks and of the importance of the separation of sub lesions at the time of their creation in the determination of the probability of their combining to form a lethal lesion. This effect of sub lesion proximity on the formation of lethal lesions was incorporated in the TDRA by dividing the sensitive volume of the cell into subvolumes called sites within which sublesions may combine with one another to form an effector lesion along with consideration of the effect of random variation of the radiation energy absorption among the sites. This is referred to as the site model of the TDRA. The site model was refined by explicitly formulating the effect of distance between sub lesions on the production of effector lesions. This is referred to as the distance model of the TDRA. A description of the distance model and the relations that result has been given and will not be included here [46].

The Microdosimetric-Kinetic Model

The Microdosimetric-Kinetic (MK) model [12-14] is an elaboration of the site model of the TDRA combined with the mass action kinetic description of radiation induced cell killing proposed in the repair misrepair (RMR) [10] and Lethal Potentially Lethal (LPL) [11] models.

In the MK model, ionizing radiation is assumed to cause lesions in DNA, called Potentially Lethal Lesions (PLL), each of which may constitute the first step in the formation of a chromosome aberration. The PLL correspond to the sublesions of the TDRA. A PLL is created by passage of a single charged particle through or near a chromatin strand. PLL are probably the same as what are referred to as complex DNA lesions or multiply damaged DNA sites [47,48]. These are thought to consist of a group of broken and rearranged covalent bonds, located within a few nanometers of each other along the DNA double strand, some on one strand, some on the other, and some involving both. Most PLL will become a double strand break when the cell is lysed; the protein of the chromatin dissolved and the double stranded DNA subjected to centrifugation or electrophoresis. The number of double strand breaks measured this way will be taken as an estimate of the rate of formation of PLL per unit dose, recognizing that the rate may be higher to the extent that some PLL may not result in a measured double strand break.

The number of double strand breaks has been found to be proportional to dose at a rate of about 30 to 60 breaks created per Gy of low LET radiation per diploid mammalian genome, depending on the method used to count them. This is also expressed as 5.6×10^{-9} to 7.4×10^{-9} double strand breaks per Gy per nucleotide pair [49-51]. It is this proportionality that implies each double strand break, or PLL, arises from a single charged particle track. The 30 breaks per Gy estimate is obtained by measuring the fraction of DNA that is unbroken and as a result is retained in the well at the origin of the electrophoresis gel and assuming random position of the breaks along the chromatin DNA strand. The 60 breaks per Gy estimate is obtained by explicitly including in the calculation the number of fragments of DNA with less than 200,000 base pairs that is measured from the DNA that migrates into the gel. It is found that break production from low LET x-rays increased from 31.3 per Gy per diploid genome, with the random assumption, to 57.8 when a count of the smaller fragments was included [51]. For nitrogen ion irradiation, with LET of 97 $keV \times \mu m^{-1}$, including small fragments increases the measured break production from 20.5 to 64.8 per Gy per diploid genome. This indicates at most a 10 percent increase in the production of double strand breaks per unit dose with an increase in LET from about 2 to 100 $keV \times \mu m^{-1}$. This is within experimental error of no increase.

A PLL site on a chromatin strand is assumed to move about in a random flight pattern in response to impulses from molecular thermal motion in a manner similar to the motion of monomer units of a randomly coiled polymer in solution. Further, it is assumed movement of each PLL is forever confined to within a space in the nucleus called a domain. Confinement may be due to a combination of tethering of the chromatin strand to the nuclear matrix or membrane

and the excluded volume of other chromosomes or distant parts of the same chromosome. The size of the domain of confinement may also be influenced by the distance a PLL can travel before it is removed by repair [52]. A PLL can interact with another PLL created in the same domain. A PLL cannot interact with PLL that are created in domains other than its own.

Domains correspond to the sites proposed in the site model version of the TDRA. They are envisioned as having various shape so that they fit together to fill the space of the nucleus like pieces in a three dimensional jig saw puzzle. For convenience the domains are assumed to all have the same size in the sense that if deformed into a sphere of unit density they all would have the same diameter called *d*. The value of *d* is in principle experimentally measureable from the RBE versus LET relation (see equations 30, 31 and Figure 3) and is found to have a value in the range of about 0.5 to 1.0 micrometers for several cultured mammalian cell lines [12-14,31]. The value of *p* ranges from a few hundred to a few thousand domains per nucleus.

Because the energy absorbed from exposure to ionizing radiation is concentrated along the paths of individual charged particles that traverse the nucleus, the energy deposited in individual domains will vary randomly from domain to domain. Let *z* be a random variable equal to the energy deposited in a domain divided by the domain mass. The value of *z* has units of Gy and represents a domain dose. It is referred to as specific energy in the microdosimetry literature. The DNA content of a domain also may vary randomly from domain to domain. It will be represented by a random variable called *g*, the value of which is equal to the mass of DNA in the domain.

The concept of a domain is a theoretical ploy adopted in the TDRA and MK models as a way to account for the circumstance that the likelihood of combination of two PLL is very low or zero if they are prevented from coming into contact because of a barrier or restraint that separates them or because of the distance between them. Curiously, microscopic observation of fluoroscopically marked points on the chromatin strand of eukaryotic cells indicates such points move by apparent random flight in a seemingly confined space of about the same size as a domain [53,54].

It is assumed that the number of PLL that form in each domain from exposure to ionizing radiation is proportional to both the domain dose and the mass of DNA in the domain [55]. That is,

$$\langle x|z,g\rangle = k_d gz \qquad (14)$$

In which *x* is a random variable equal to the number of PLL created in a domain from exposure to ionizing radiation and ‹*x*|*z*, *g*› is the average of *x* over the subset of domains that contain *g* gm of DNA and have absorbed a dose of *z* Gy. Let *k* be the number of PLL created per cell per Gy, estimated, as noted above, as about 40. The value of k_d is determined by the relation,

$$k = p\langle g\rangle k_d \qquad (15)$$

Note that $p\langle g\rangle$ equals the total DNA content of the nucleus. The validity of equation 14 is supported by the observation of proportionality between dose and double strand break production.

1. A PLL is assumed to undergo any one of four processes that correspond to those proposed in the repair misrepair [10] and lethal potentially lethal models [11]. These are:It may be repaired by a monomolecular-like (monolesional) process governed by mass action rate constant *c*. The value of *c* is equal to the probability per unit time of disappearance of the PLL by repair. The value of *c* is the order of 1.0 hr^{-1} for many mammalian cells [56]. Repair preserves or restores the continuity of the chromatin DNA strand at the PLL site. It is carried out predominantly by the error prone Non Homologous End Joining (NHEJ) process which is expected to introduce incorrect base sequence or deletion of several base pairs at each repair site. Note: the configuration of the chemical bonds at a PLL site may vary from PLL to PLL. The repair rate may be significantly different for PLL with different configurations, leading to multiple values of *c*. If this is the case, the single value used here, to avoid confusing mathematical complexity, is to be regarded as an appropriately weighted average. If the value of *c* has significant variation among the PLL repair sites, the average will have its maximum value immediately after an instantaneous exposure to radiation and decrease over time to monotonically approach a minimum value as the PLL that repair faster are eliminated.

2. A PLL may convert to a lethal unrepairable lesion by a monolesional process with rate constant *a*. This may be by separation of the chromatin strand of DNA into two fragments, one that contains a centromere and one that is acentric. This may occur while the PLL is waiting to be repaired or during the repair process. The sensitivity of mammalian cells, as discussed further below, is such that the value of *a* can be at most a few percent of the value of *c* so that $(a+c) \approx c$ [12]. The value of *a*, like that of *c*, may be an average.

3. A PLL may combine with a second PLL that has formed in the same domain, to form a lethal unrepairable lesion by misrepair linkage of the DNA double strand on one side of one PLL to that on one side of the other PLL and reciprocal linkage of the other two participating chromatin strands. This will result in a lethal dicentric chromosome and an acentric fragment (asymmetric exchange) or a translocation aberration that is not necessarily lethal (symmetric exchange). If the exchange is incomplete in that one of the pair of strands fails to join, lethal acentric fragments result. The rate constant for the formation of a lethal lesion by this bilesional reaction is called b_d. The value of b_d equals one half of the probability per unit time of a PLL undergoing a lethal bilesional reaction when there is on average one PLL per domain. Note that an exchange type of chromosome aberration may also occur if a PLL interacts with a site on a nearby chromatin strand that has no radiation induced PLL in a way that leads to an exchange of strands, complete or incomplete. If this occurs it would contribute to the value of the monolesional rate constant *a* rather than to the bilesional rate constant b_d.

4. If a PLL undergoes none of the above reactions and persists for a time period t_r after its creation, it becomes lethal and unrepairable. For instance, this may come about because of the replication of DNA on either side of the PLL creating a gap in the chromatin strand that persists until the next mitosis leading to an acentric fragment. The damage recognition and cell cycle control systems in a cell identify PLL, delay progress through the cell cycle and delay entry into mitosis until each PLL is repaired or converted to a lethal unrepairable lesion by one of the processes governed by the rate constants *a, b* and *c*. If a PLL is not able to be recognized or processed by one of these reactions t_r will be the time before the cell reaches mitosis. This may be as short as an hour for a cell in G2 phase that has passed the time of G2 arrest or as long as 10 hr to 20 hr for a cell in G1 or early S phase. If the cell is in a quiescent phase and not cycling t_r will include the time before it enters the cycle and initiates DNA synthesis. If the damage recognition, cell cycle control and repair functions work perfectly to

delay mitosis until the PLL is removed t_r will be effectively infinite. Note that allowing for a finite t_r makes it unnecessary to postulate a radiation induced lesion in DNA that is unrepairable and inevitably lethal.

$$\begin{aligned}\langle w\rangle &= \alpha D + \beta D^2 \\ &= (\alpha_0 + \gamma\beta) D + \beta D^2\end{aligned}$$

Consider exposure of a population of mammalian cells to dose D of ionizing radiation over a time span much shorter than the half life of a PLL, so as to be effectively instantaneous. Let x be a random variable equal to the number of PLL in a domain and ‹$x|z, g$› be the average of x over the subset of domains that have DNA content g and absorbed a dose equal to z. Let ‹$w_d|z, g$› be the average number of lethal lesions that form in the same subset of domains. The mass action kinetic equations that govern the time course of these averages are, with a dot over a symbol indicating differentiation with respect to time;

$$\begin{aligned}\langle \dot{x}|z,g\rangle &= -(a+c)\langle x|z,g\rangle - 2b_d \langle x|z,g\rangle^2 \\ &\approx -(a+c)\langle x|z,g\rangle\end{aligned} \tag{16}$$

$$\langle \dot{w}_d|z,g\rangle = a\langle x|z,g\rangle + b_d\langle x|z,g\rangle^2 \tag{17}$$

The approximation of the second line of equation 16 is possible because the most likely fate of a PLL is, by far, that it will be repaired. It can be shown [12] that the approximation is valid for instantaneous dose much less than $k/4\beta$. For $k \approx 40$ PLL per Gy per diploid mammalian genome and $\beta \approx 0.05$ Gy^{-2} this is dose much less than 200 Gy. It can be shown that for cells with $\alpha \approx 1.0$ Gy^{-1} the value of $a/c < 0.02$ and as the value of α decrease the maximum possible value of a/c decreases. Thus $(a+c)$ can be replaced by c for nearly all cells [12].

The solution of approximate form of equation 16 is,

$$\langle x|z,g\rangle = k_d g z e^{-(a+c)t} \tag{18}$$

Insert ‹$x|z, g$› from equation 18 into equation 17, integrate from zero to t_r and add the lethal lesions from persistence of PLL to t_r to obtain,

$$\langle w_d|z,g\rangle = Agz + Bg^2z^2 \tag{19}$$

In which,

$$A = \frac{ak_d}{(a+c)} + k_d e^{-(a+c)t_r} \text{ and } B = \frac{b_d k_d^2}{2(a+c)}\left(1 - e^{-2(a+c)t_r}\right) \tag{20}$$

Computation of the average of equation 19 over all values of g and z leads to an equation for the average number of lethal lesions per cell ‹w› [12-14],

$$\begin{aligned}\langle w\rangle &= \alpha D + \beta D^2 \\ &= (\alpha_0 + \gamma\beta) D + \beta D^2\end{aligned} \tag{21}$$

For exposure to radiation with LET less than about 70 $keV \times \mu m^{-1}$ the lethal lesions are Poisson distributed among the cells of the irradiated population so that ‹w› = -ln$S$ and this becomes the linear quadratic survival relation,

$$\begin{aligned}-\ln S &= (\alpha_0 + \gamma\beta) D + \beta D^2 \\ &= \alpha D + \beta D^2\end{aligned} \text{ with } \alpha = (\alpha_0 + \gamma\beta) \tag{22}$$

The value of γ has the dimension of dose and is given by,

$$\gamma = \frac{\langle z_1^2\rangle}{\langle z_1\rangle} = \frac{\delta^2 z}{D} \tag{23}$$

The value of z_1 is a random variable equal to the dose imparted to a domain by the passage of a single charged particle through or near it. The ratio ‹z_1^2›/‹z_1› is the dose weighted average of the distribution of z_1 and $\delta^2 z_\infty$ is the variance of the distribution of the accumulated domain dose z. Further [13,14]

$$\begin{aligned}\gamma &= \frac{0.203 y_D}{d^2} \text{ and } \gamma = \frac{\langle E^2\rangle}{m\langle E\rangle} + \frac{0.229 L_D}{d^2} \\ &\approx \frac{0.229 L_D}{d^2}\end{aligned} \tag{24}$$

The decimal numbers in equation 24 are such that the units of γ are Gy if those of L_D and y_D are in $keV \times \mu m^{-1}$ and the domain diameter is in μm. The value of L_D is the dose weighted average of the track average linear energy transfer (LET). The value of E is a random variable equal to the energy lost at each of the points along the trajectory of the radiation induced high energy charged particle at which there is an energy transfer to an electron of the exposed tissue or cells. The value of m is the mass of a domain. The value of the term containing E in equation 24 is estimated to be about 0.032 Gy [13]. As indicated, it can generally be neglected. The value of L_D requires specification of a delta ray cut off energy. If a delta ray cut off energy is not specified it usually refers to infinite energy cut off which is the stopping power. It is not a directly measureable property of a radiation beam. Its value must be calculated [57].

On the other hand, the value of y_D is the dose weighted average of the lineal energy density of the radiation beam [58]. It is a microdosimetric quantity measureable with a tissue equivalent proportional counter (TEPC) with effective diameter approximately equal to the domain diameter d [58]. As discussed below, the equivalent sphere diameter of the domain of several mammalian cell lines has been estimated, from the dependence of RBE on LET or y_D, to be in the range of about 0.5 to 1.0 μm. Happily, the value of y_D is only weakly dependent on the equivalent diameter of the TEPC used to measure it [58], so that selection of a specific equivalent diameter of the TEPC in this range for the measurement of y_D does not significantly affect the value of y_D or the calculation of γ from equation 24. On the other hand, the value of L_D is not a directly measurable property of a beam of radiation. Its value is the result of a complicated calculation requiring a number of assumptions including the assignment of a delta ray cut off energy. This suggests characterization of the quality of a beam of ionizing radiation, that we refer to generically as its "LET", for the purpose of interpreting the result of radiobiologic experiments, or for treatment planning of hadron particle beam therapy, is better represented by the measureable quantity y_D than by calculated estimates of L_D.

The other constants in equation 22 are,

$$\begin{aligned}\alpha_0 &= \frac{ak}{(a+c)}\left(1 - e^{-(a+c)t_r}\right) + ke^{-(a+c)t_r} \\ &\approx \frac{ak}{(a+c)} + ke^{-(a+c)t_r}\end{aligned} \tag{25}$$

$$\begin{aligned}\beta &= \frac{bk^2}{2(a+c)}\frac{\langle g^2\rangle}{\langle g\rangle^2}\left(1 - e^{-2(a+c)t_r}\right) \\ &\approx \frac{bk^2}{2(a+c)}\frac{\langle g^2\rangle}{\langle g\rangle^2}\end{aligned} \tag{26}$$

The value of $k=p\ \langle g\rangle\ k_d$ is the average number of PLL created per Gy per diploid mammalian cell genome. Note that $p\ \langle g\rangle$ is the mass of DNA in the nucleus. The value of k equals about 40 [49-51]. The value of $b=b_d/p$ is the probability per unit time of two PLL combining to form a lethal lesion when there is on average one PLL per cell. The value b is the order of $10^{-4}\ h^{-1}$ which is about 0.01 percent of c [12].

An estimate of the effective value of $(a+c)t_r$ and t_r can be obtained from rearrangement of equation 25,

$$(a+c)t_r = -\ln\left[\frac{\alpha_0 - \frac{ak}{(a+c)}}{k}\right] = -\ln\left[\frac{\alpha_0}{k} - \frac{a}{(a+c)}\right] = -\ln\left[\frac{\alpha_R - \gamma\beta_R}{k} - \frac{a}{(a+c)}\right] \quad (27)$$

High energy x-rays and Cobalt-60 gamma rays have $y_D \approx 2.0\ keV\times\mu m^{-1}$ [58] so, per equation 24, the value of γ ranges from about 1.0 to 1.6 Gy as the diameter of a domain ranges from about 0.75 to 0.5 μm. Setting $\gamma \approx 1.3$ Gy for reference low LET radiation and $\beta_R = 0.05\ Gy^{-2}$ the value of $\gamma\beta_R \approx 0.065\ Gy^{-1}$. Values of β_R as low as 0.01 Gy^{-2} have been measured for some cells. This gives a value of $\gamma\beta = 0.013\ Gy^{-1}$. Inserting the average of these values in equation 27, and noting that $(a+c) \approx 1.0\ h^{-1}$ and that $a/(a+c) \approx 0.01$ or less [12],

$$(a+c)t_r \approx -\ln\left[\frac{\alpha_R - 0.04}{k} - \frac{a}{(a+c)}\right] \approx -\ln\left[\frac{\alpha_R - 0.04}{k} - 0.01\right] \quad (28)$$

The logarithm of a negative quantity is not defined and that of zero is infinity. Therefore, if $(\alpha_R - 0.04)/k$ is less than 0.01, given the uncertainty in the values of α_R, k and $a/(a+c)$, $(a+c)t_r$ and t_r are likely very large and effectively infinite. Thus, if α_R is clearly less than about 0.36 Gy^{-1}, for instance 0.25 Gy^{-1} or less, the value of t_r is likely a very long time and effectively infinite.

Malignant cells of carcinomas, sarcomas and gliomas resemble those of the immortalized cell lines V79, T-1 and HSG and like them generally have α_R less than 0.25 Gy^{-1} [31-36] suggesting a value of effectively infinite t_r for these cancers.

The value of α_R of cultured normal skin fibroblast GMO5389 [36] and HF-19 [59] is, respectively, 0.70 and 0.80 Gy^{-1}. Unlike those of V-79, T-1 and HSG, these cells are not immortalized. Equation 28 indicates a value of $(a+c)t_r \approx 5$ and $t_r \approx 5$ hours. Note that cells with this high a value of α may have $a/(a+c)$ greater than 0.01. This would increase the value of t_r calculated from equation 28. For instance, if $a/(a+c)$ were 0.02 instead of 0.01 equation 28 indicates effectively infinite t_r.

As noted above, the values of α_R of epithelial stem cells irradiated *in situ* in animals are estimated as between about 0.1 and 0.2 $Gy^{-1}$for several tissues corresponding to infinite t_r. Bone marrow stem cells have α_R of about 1.0 Gy^{-1} corresponding [23], per equation 28, to a value of $(a+c)t_r \approx 5$. The ataxia telangiectasia skin fibroblasts have the ATM mutation and are deficient in damage recognition and cell cycle control function. They have α_R=2.5 Gy^{-1} [59,60]. Per equation 28 this corresponds to $(a+c)t_r \approx 3$.

Equating the two expressions for γ found in equation 24 and solving for L_D defines a value of L_D that is consistent with the experimental quantity y_D. This is,

$$L_D \approx 0.91 y_D \quad (29)$$

Dependence of RBE on Linear Energy Transfer: Linear Range

Let RBE_S be the ratio D_R/D_H, where D_H is the dose of a high LET radiation that produces the same surviving fraction S as dose D_R of a reference low LET radiation, usually high energy x-rays or Cobalt-60 gamma rays. For linear quadratic survival, the RBE in the limit of survival fraction equal to one (RBE_1) is the ratio α/α_R where α_R is the linear survival constant of the reference radiation and α is that of the higher LET radiation (see equation 46) From equations 21 and 24,

$$RBE_1 = \frac{\alpha_0 + \gamma\beta}{\alpha_R} = \frac{\alpha_0}{\alpha_R} + \left(\frac{\langle E^2\rangle}{m\langle E\rangle} + \frac{0.229}{d^2}L_D\right)\frac{\beta}{\alpha_R} = \left(\frac{\alpha_0}{\alpha_R} + \frac{\langle E^2\rangle}{m\langle E\rangle}\frac{\beta}{\alpha_R}\right) + \frac{0.229}{d^2}\frac{\beta}{\alpha_R}L_D \approx \frac{\alpha_0}{\alpha_R} + \frac{0.229}{d^2}\frac{\beta}{\alpha_R}L_D \quad (30)$$

Alternatively, using the measureable microdosimetric quantity y_D to express the LET,

$$RBE_1 = \frac{\alpha_0}{\alpha_R} + \frac{0.203}{d^2}\frac{\beta}{\alpha_R}y_D \quad (31)$$

Experiments in which RBE has been measured as a function of LET usually have reported the results using a track segment value for L_D [32-35,59-60]. The delta ray cut off energy is not stated but is implied to be infinite. One study has reported results with LET given as a measured value of y_D [36].

As illustrated in Figure 3, RBE_1 increases linearly with increasing LET for LET less than about 50 to 100 $keV \times \mu m^{-1}$. The linearity of the experimentally determined RBE_1 versus LET relation is evident in several other RBE_1 versus LET studies [12-14,31]. It is the most striking and significant finding of these experiments. It is obscured by the practice of plotting LET on a logarithmic axis. Per equations 30 and 31, the linearity implies the value of the ratio β/d^2 does not vary significantly with increasing LET in this range. The rate of increase of RBE_1 with LET in this range of LET is proportional to the ratio β_R/α_R and to the reciprocal of the square of the domain diameter. As noted above, there is experimental evidence that β does not change significantly with variation in LET, at least for LET less than about 70 $keV \times \mu m^{-1}$, and possibly beyond [43-45]. This suggests that it is not only the ratio β/d^2 that is constant in this range but also the values of β and domain diameter d. The dependence of β on the kinetic constants (equation 26) suggests invariance of the values of k and c for LET upto about 70 $keV \times \mu m^{-1}$, and possibly beyond.

Per equation 31, if LET is expressed as y_D, the intercept at zero of the extrapolation of the line determined by the experimental measurement of RBE_1 is equal to α_0/α_R. The value of α_0/α_R is the proportion of lethal lesions produced by the reference low LET radiation that arise from a single PLL. One minus α_0/α_R is the proportion that arises from binary combination of PLL.

Per equation 30, if LET is expressed as L_D the zero LET intercept includes a term that contains the ratio $\langle E^2\rangle/m\langle E\rangle$, estimated as about 0.023 Gy [17]. The ratio β/α is generally less than 0.2 Gy^{-1} so that this term will be the order of 0.005 or less, which is less than the experimental error of determination of the intercept.

If the value of the α/β ratio for reference low LET radiation is known, the diameter of the domain d can be obtained from the slope

of the RBE_1 line. Experimental studies are available for several cell lines that allow determination of a value of d [31]. For HSG cells d equals 0.89 μm [36]; for T-1 cells d equals 0.75 μm [32]; for V79 cells synchronized to be at the G1/S transition of the cell cycle, d equals 0.61 μm and for V79 cells synchronized to be in late S phase d equals 0.56 μm [33].

For radiosensitive cells, for instance those for which α is greater than about 0.5 Gy^{-1}, the value of β has not been able to be measured because of the dominance of α in the survival relation. Examples are diploid human skin fibroblasts [36,61] including from patients with ataxia telangiectasia [59,60]. Because of the relatively large value of α_R for these cells, the ratio β/α_R is expected to be relatively small. This is reflected in the low rate of increase in RBE_1 with increasing LET observed for cells relatively sensitive to low LET photon radiation [36,59-61].

The composition and structure of chromatin in the interphase nuclei of mammalian cells does not vary much from cell type to cell type. The domain diameter in the radiosensitive and other mammalian cells likely does not vary greatly from the range spanned by d in the cells for which the domain diameter is measureable. The value of d in all, or nearly all, mammalian cells is expected to be in the range of about 0.5 μm to 1.0 μm.

Another quantity of interest that can be determined from the slope of the RBE_1 versus LET line is the ratio β/d^2. Since $(a+c)$ essentially equals c, β is proportional to c^{-1}. As has been discussed [52], d^2 will be proportional to c^{-1} if its value is determined by the average of the straight line distance between the origin and terminus of the random flight path followed by a PLL until it is removed by repair. Thus, to the degree that the random flight of a PLL determines d, the ratio β/d^2 will be independent of the value of c. If this is the case, cells with widely different sensitivity to low LET irradiation related to different values of c, would tend to have less variability in the value of β/d^2. Among a group of 7 cell lines for which α_R varies from 0.06 to 2.5 Gy^{-1} the average value of β/d^2 equals 0.085 and varies only from 0.053 to 0.13 $(Gy \times \mu m)^{-2}$ [31]. The variation does not appear related to that of α_R.

This relatively low variation of β/d^2 among cells with vastly different sensitivity to low LET radiation suggests that the length of the random flight of a PLL has some influence on the size of the domain. It also suggests that absent a better way, an estimate of RBE_1 for a hadron radiation beam with LET less than about 80 $keV \times \mu m^{-1}$ can be made by using the average value of β/d^2 equal 0.10 $(Gy \times \mu m)^{-2}$. This is, from equations 30 and 31, substituting 0.10 for β/d^2, neglecting the small value of α_0/α_R and making RBE_1 for the reference radiation equal to one,

$$RBE_1 = 1+\frac{\beta_R}{\alpha_R}\frac{0.203}{d^2}(y_D - y_{DR}) \approx 1+0.414\frac{\beta_R}{\alpha_R}(y_D - y_{DR}) \approx 1+\frac{0.0172}{\alpha_R}(y_D - y_{DR})$$

$$= 1+\frac{\beta_R}{\alpha_R}\frac{0.229}{d^2}(L_D - L_{DR}) \approx 1+0.467\frac{\beta_R}{\alpha_R}(L_D - L_{DR}) \approx 1+\frac{0.0195}{\alpha_R}(y_D - y_{DR}) \quad (32)$$

In which y_{DR} and L_{DR} refer to LET of the reference radiation. For Cobalt-60 gamma radiation y_{DR}=1.8 $keV \times \mu m^{-1}$ and for 250 kVp xrays is y_{DR}=4.7 $keV \times \mu m^{-1}$. The first approximate relation is with the domain diameter assigned an average value found for 4 cell lines that is d=0.7 μm [31]. The second approximate relation is with the ratio β_R/d^2 assigned an average value of 0.085 that was found for 7 cell lines [31].

Note that the near equality relation 32 illustrates the important generalization that the RBE for cell reproductive death for cells more sensitive to low LET radiation, as expressed in a relatively large value of α_R, increases less with increasing LET than that of cells that are less sensitive to low LET radiation. That is, radio sensitivity differences between mammalian cells tend to decrease in a predictable way as LET increases for LET up to about 75 $keV \times \mu m^{-1}$. This is the range of LET of most importance in the application of hadron irradiation in clinical radiation oncology.

The value of α_R of two diploid human fibroblast cell lines is 0.7 and 0.8 Gy^{-1} [31,36,61]. In contrast, the value of α_R of immortalized, aneuploid cell lines derived from malignant tumors or fetal tissue is in the range of about 0.1 Gy^{-1} to 0.3 Gy^{-1} [31]. This suggests malignant cells may tend to be less sensitive to radiation than normal tissue cells, particularly those stem cells that form and maintain connective tissue. Radiation injury to connective tissue is implicated as one of the causes of late developing radiation injury to normal organs. The risk of late developing radiation injury to normal organs is often what limits the dose prescribed for radiation treatment with curative intent. This suggests there may be a radiobiological advantage to irradiation with higher LET hadron beams from decrease in the difference between the radio sensitivity of the cells of normal and malignant tissues as LET increases.

The survival of the radio resistant cells of a targeted heterogeneous tumor cell population can be an important reason for failure of a course of radiation to cure a cancer. Decrease in the difference between the value of α of a radio resistant component and that of the average value of α of a heterogeneous tumor cell population is another advantage to treatment with higher LET hadron radiation. An important example is the resistant hypoxic component in many malignant tumors [31].

Effect of Non Poisson Distribution of Lethal Lesions at LET Greater than that of Linear Range

As illustrated in Figure 3 the increase in experimentally measured RBE_1 with increasing LET becomes progressively less than that of the linear increase established for the range of LET less than about 70 $keV \times \mu m^{-1}$. The slope of the RBE versus LET relation eventually becomes negative so as to form a maximum around 100 $keV \times \mu m^{-1}$. This could be due to a decrease in the production of PLL per unit dose, an increase in the rate of repair of PLL or deviation of the distribution of lethal lesions among the population of irradiated cells from that of the Poisson distribution. The effect of deviation from the Poisson distribution is the subject of this section and appears to be the most important, if not the only, reason for the development of a maximum in the experimental RBE_1 curve [14].

The tracks of charged particles produced in matter by exposure to ionizing radiation are randomly distributed in space so that the number of tracks that pass through or near the DNA of a mammalian cell nucleus is Poisson distributed. If each track never, or hardly ever, causes two or more lethal lesions in the cell it traverses, the lethal lesions will also be distributed randomly according to the Poisson distribution. If there is a significant chance of a single track causing two or more lethal lesions the fraction of cells that will have more than one lethal lesion will be greater than that indicated by the Poisson distribution and the fraction of cells that survive with no lethal lesions will be greater. The excess survival from this clustering

of lethal lesions in some cells and the resultant sparing of others will be more significant the lower the dose and most pronounced in the limit of zero dose. The greater the LET of the radiation, the great the chance of producing more than one lethal lesion from a single particle track.

Let the sensitive nuclear volume be that part of the nucleus in which ion pairs produced by the track of a charged particle can cause a PLL in the DNA. The sensitive volume will consist of the DNA and associated chromatin protein and solvent that is in contact with, or so near to, the DNA that ionized molecules, ejected electrons and resultant free radical intermediates can cause a PLL.

The nucleus of a small diploid human lymphocyte in the G0 phase of the cell cycle is among the most compact of mammalian cell nuclei and on light microscopy it appears to be filled with a uniform density of DNA. It contains about 6 pg of DNA in a volume equal to that of a 5 μm diameter sphere to make a concentration of DNA of about 0.1 pg per μm^{-3} (0.1 gm/ml). Using this as an estimate of the concentration of DNA in the sensitive nuclear volume indicates the mass of the sensitive nuclear volume is about 10 times the mass of the DNA contained in the nucleus.

Let the dose to the sensitive nuclear volume from a single charged particle track through the nucleus be γ_N. The value of γ_N can be estimated as,

$$\gamma_N = \frac{0.16hL}{M} \tag{33}$$

In which h is the average length in μm of the intersection of the path of the charged particle with the sensitive nuclear volume as it passes through the nucleus; L is the LET from the particle along its path in $keV \times \mu m^{-1}$ and M is the mass of the sensitive nuclear volume in pg. The decimal constant is determined by the units so that γ_N is in Gy. The sensitive nuclear volume has no definable shape but can be thought of as gathered together to form a cylinder of unit density with cross sectional area σ and height equal to h. The cylinder is thought of as oriented with its axis parallel to the direction of the beam of charged particles so that h has the same value as in equation 33. Since the density of the cylinder is one, M equals the product $h\sigma$. Then γ_N can be rewritten as,

$$\gamma_N = \frac{0.16}{\sigma}L \tag{34}$$

with σ in μm^2. The value of the cross section σ will be similar, but not necessarily equal, to the cross sectional area of the nucleus as visualized with a light microscope. The factor relating σ to the nuclear cross section will be determined by the distribution of the chromatin in the nucleus and the thickness of the nucleus in the direction of the radiation beam. A value can be determined for σ by fitting equation 43 or 44 (see below) to measurements of RBE_1 as LET is increased to exceed that of the linear range.

Let Φ be the fraction of cells that suffer a single charged particle traversal (a single event) after exposure to dose D where D is so low that the chance of a cell hosting two or more events is negligible. Let $\langle w_N|\gamma_N\rangle$ be the number of lethal lesions created per nucleus averaged over those cells that suffer a single event. Then, since there is only one particle track through the nucleus all lethal lesions must arise from PLL of a single track.

$$\begin{aligned}\langle w_N|\gamma_N\rangle &= \alpha_P\gamma_N \qquad \text{with } \alpha_P = (\alpha_0+\gamma\beta)\\ &= (\alpha_0+\gamma\beta)\gamma_N\end{aligned} \tag{35}$$

The value of $\langle w_N|\gamma_N\rangle$ is an estimate of the average number of lethal lesions in a cell that has suffered one event. The subscript on α_P indicates it is the value of the linear survival constant that will be found, per equation 30 or 31, if the lethal lesions are Poisson distributed in actuality, as they are for low LET radiation, or if they were to be magically redistributed to be in a Poisson distribution for the higher LET radiation.

Let S_1 be the surviving fraction of those cells that suffer a single event. Lethal lesions will be distributed by the Poisson distribution among the cells that have had a single event. It follows that

$$\begin{aligned}S_1 &= e^{-\langle w_N|\gamma_N\rangle}\\ &= e^{-(\alpha_0+\gamma\beta)\gamma_N}\end{aligned} \tag{36}$$

The fraction of the whole population that survive is then,

$$\begin{aligned}S &= (1-\Phi)+\Phi S_1\\ &= (1-\Phi)+\Phi e^{-(\alpha_0+\gamma\beta)\gamma_N}\end{aligned} \tag{37}$$

The average number of lethal lesions per cell averaged over all cells is

$$\langle w_N\rangle = \Phi\langle w_N|\gamma_N\rangle \tag{38}$$

Substituting for Φ equation 38 in equation 37 gives,

$$S = 1+\frac{\langle w_N\rangle}{\langle w_N|\gamma_N\rangle}\left(e^{-(\alpha_0+\gamma\beta)\gamma_N}-1\right) \tag{39}$$

Substituting for $\langle w_N\rangle$ from equation 21 gives,

$$\begin{aligned}S &= 1+\frac{\left(e^{-(\alpha_0+\gamma\beta)\gamma_N}-1\right)}{(\alpha_0+\gamma\beta)\gamma_N}\left[(\alpha_0+\gamma\beta)D+\beta D^2\right]\\ &= 1+\frac{\left(e^{-\alpha_P\gamma_N}-1\right)}{\alpha_P\gamma_N}\left[\alpha_P D+\beta D^2\right]\end{aligned} \tag{40}$$

Take the natural log of S, expand in a Taylor series around D equal zero and drop terms proportional to the square or higher power of D to obtain for the limit as the dose decreases to zero,

$$\begin{aligned}\ln S &= -\frac{\left(1-e^{-\alpha_P\gamma_N}\right)}{\alpha_P\gamma_N}\alpha_P D \text{ with } \alpha = \frac{\left(1-e^{-\alpha_P\gamma_N}\right)}{\alpha_P\gamma_N}\alpha_P\\ &= -\alpha D\end{aligned} \tag{41}$$

As LET increases and the distribution of lethal lesions deviates from that of the Poisson distribution, the experimentally measured linear survival coefficient α decreases from the value of α_P as indicated by the factor in equation 41. Note that in equations 41, as LET decrease the value of α approaches that of α_P.

Form RBE_1 equal to α/α_R with α as given in equation 41,

$$\begin{aligned}RBE_1 &= \frac{\left(1-e^{-\alpha_P\gamma_N}\right)}{\alpha_P\gamma_N}\frac{\alpha_P}{\alpha_R}\\ &= \frac{\left(1-e^{-\alpha_P\gamma_N}\right)}{\alpha_P\gamma_N}RBE_{1P}\end{aligned} \tag{42}$$

The value of RBE_{1P} is RBE in the limit of zero dose that is found when the lethal lesions are either actually Poisson distributed, as they are for the lower LET range, or magically redistributed so as to be Poisson distributed as LET increases to greater than about 70 to 100 $keV \times \mu m^{-1}$.

Introduce the expressions for γ and γ_N of equation 33 into

equation 42 to obtain,

$$RBE_1 = \frac{\left(1-e^{-\alpha_P \frac{0.16}{\sigma}L}\right)}{\alpha_R RBE_{1P}\frac{0.16}{\sigma}L} RBE_{1P} = \frac{\left(1-e^{-\alpha_P \frac{0.16}{\sigma}L}\right)}{\alpha_R \frac{0.16}{\sigma}L} \quad (43)$$

A term with the negligible value of $\langle E^2\rangle/m\langle E\rangle$ has been left out of equation 43. Note there is no subscript on L to indicate a delta ray cut off energy. The value of L without subscript is to be taken as that value consistent with the measured value of y_D per equation 29. The cutoff of delta ray energy appropriate to L is expected to be close to that of the energy of a delta ray electron sufficient to propel it a distance equal to the diameter of a domain.

Alternatively, using y_D for the dependence of γ on LET,

$$RBE_1 = \frac{\left(1-e^{-\alpha_P \frac{0.14}{\sigma}y_D}\right)}{\alpha_R RBE_{1P}\frac{0.14}{\sigma}y_D} RBE_{1P} = \frac{\left(1-e^{-\alpha_P \frac{0.14}{\sigma}y_D}\right)}{\alpha_R \frac{0.14}{\sigma}y_D} \quad (44)$$

Note that as y_D or L decrease toward zero, and terms in y_D or L squared or higher order become negligible, RBE_1 approaches RBE_{1P}. As y_D or L become large RBE_1 approaches $\sigma \times (0.14\alpha_R y_D)^{-1}$. The variation of RBE_1 with increasing LET is such that for LET less than about 70 $keV \times \mu m^{-1}$ it increases linearly with increasing LET with a slope proportional to β_R/α_R. For LET more than about 150 $keV \times \mu m^{-1}$ RBE_1 decreases at a rate proportional to the ratio σ/α_R and to the reciprocal of the LET.

The LET at which RBE_1 reaches its maximum value is that at which the derivative of RBE_1 with respect to y_D is zero. This can be shown to be y_D^{max},

$$y_D^{\max} \approx 8.4\sqrt{\sigma}\sqrt{\frac{d^2}{\beta_R}} \quad (45)$$

Substituting into equation 44 gives an estimate of the maximum reached by RBE_1. This is,

$$RBE_1^{\max} \approx 0.74\frac{\sqrt{\sigma}}{\alpha_R}\sqrt{\frac{\beta_R}{d^2}} \approx 0.74\frac{\sqrt{\sigma}}{d\sqrt{\beta_R}}\left(\frac{\beta_R}{\alpha_R}\right) \quad (46)$$

An estimate of the value of σ is obtained from the fit of the experimental RBE_1 to equation 43 or 44. To do this, RBE_{1P} is first determined by fitting a straight line to the experimental RBE_1 in the linear range, i.e., below about 70 $keV \times \mu m^{-1}$. With the linear relation for RBE_{1P} and the related linear relation for α_P established, RBE_1 as a function of y_D or L is plotted using equation 43 or 44 for various trial values of σ as shown in Figure 3 [14].

The value of σ estimated in this manner is a property of the cell and its physiologic state. It is expected to be less than that of the nuclear cross section measured with a light microscope due to the sensitive nuclear volume being less than that of the nucleus for most cells, and because the trajectory of a charged particle through a thin interphase cell spread on the surface of a culture dish will sometimes not produce a lethal lesion. The value of σ is expected to depend on nuclear features that vary with experimental parameters of a cell culture or cell environment in a tissue or tumor. For instance, σ may be affected by, among other things, cell population density, phase of the cell cycle, cell metabolic activity, cell hydration, oxygenation and nuclear thickness and shape. If the nucleus is not spherically symmetrical it may depend on orientation of the cell relative to the radiation beam direction. These features are dependent on details of cell culture that are difficult to control and reproduce. In general, as shown in several RBE_1 versus LET studies [14,31] there is less random variation of measured values of RBE_1 in the lower LET range where RBE_1 equals RBE_{1P} and cell survival is not significantly affected by σ than as LET is increased to where RBE_1 is affected and eventually dominated by σ.

Dependence of RBE on Survival Level

The emphasis here has been on the RBE in the limit of survival fraction equal to one, that is, RBE_1. Consider now the RBE at survival level S, called RBE_S. Let subscript R designate low LET reference radiation quantities and unsubscripted quantities designate high LET quantities. Then, noting that the definition of RBE_S is $RBE_S=D_R/D$ where D_R and D each give the same linear quadratic surviving fraction equal to S. The definition of RBE_S implies,

$$\alpha_R D_R + \beta_R D_R^2 = \alpha D + \beta D^2 \quad (47)$$

Divide through by D and noting that $\alpha=\alpha_R RBE_1$ gives a quadratic equation for RBE_S

$$\alpha_R RBE_S + \beta_R RBE_S^2 D = \alpha_R RBE_1 + \beta D \quad (48)$$

The solution is,

$$\begin{aligned} RBE_S &= \frac{-\alpha_R + \sqrt{\alpha_R^2 + 4\beta_R D(\alpha_R RBE_1 + \beta D)}}{2\beta_R D} \\ &= \frac{-\alpha_R + \alpha_R\sqrt{1+4\beta_R D\left(\frac{RBE_1}{\alpha_R}+\frac{\beta D}{\alpha_R^2}\right)}}{2\beta_R D} \\ &= \frac{\alpha_R}{2D\beta_R}\left[-1+\sqrt{1+4\frac{\beta_R}{\alpha_R}D\left(RBE_1+\frac{\beta D}{\alpha_R}\right)}\right] \end{aligned} \quad (49)$$

The limit of this expression for RBE_S approaches RBE_1 as D approaches zero and the surviving fraction approaches one. The limit of RBE_S approaches $\sqrt{\beta/\beta_R}$ as D becomes very large. It has been pointed out that RBE_1 is the maximum of RBE_S and $\sqrt{\beta/\beta_R}$ is its minimum [66]. For the range of LET for which RBE_1 is proportional to LET, and likely as LET exceeds that range, $\beta=\beta_R$ so that the minimum value of RBE_S is one.

Biophysical Models and Clinical Radiation Oncology

The clinical effect of the exposure of normal and targeted neoplastic tissue to ionizing radiation is determined by the fraction of the cells that survive. This in turn will be determined by clinical parameters of treatment including the absorbed dose, the schedule with which it is administered and the identity of the ionizing charged particle. The biophysical models discussed here provide a way to quantitatively express the biological and clinical effect through definition of $BED_{\alpha/\beta}$ and RBE_S. The models identify and define the several variables on which $BED_{\alpha/\beta}$ and RBE_S depend and relate them to the clinical parameters of treatment. These variables include the survival constants α and β, and particularly their ratio, the repair half life of the potentially lethal lesions in DNA, the doubling time of the exposed cell population, the domain diameter and the LET quality of the treatment beam. If the radiation has LET greater than about 60 to 80 $keV \times \mu m^{-1}$ there is significant dependence of cell survival on the value of the cross section σ. The value of σ is determined by the size and shape of the irradiated cell nucleus, the distribution of

its contained chromatin and its orientation to the direction of the treatment beam.

When there is limited experience to guide selection of the clinical parameters of treatment, or if optimization of these parameters is being studied in a clinical trial, biophysical models have been helpful. The $BED_{\alpha/\beta}$ as shown in equation 15 is widely used to compare the effect of fractionated courses of radiation treatment including in clinical trials. Its use has been extended to provide a measure of the effectiveness of continuous irradiation exposure *via* implantation or targeted incorporation of radionuclides, including alpha particle emitters that have a high LET [64-68]. The estimation of RBE_S is an important issue in planning treatment with alpha particle emitting nuclides and in treatment with heavy particle irradiation, notably with protons and carbon ions [31,36,69-72]. In estimating the effect of a new treatment, particularly one intended to exploit an advantage of increased linear energy transfer radiation; biophysical models furnish a way to appreciate the factors that influence the level of the effect of LET on cell survival and the degree of uncertainty in their value.

Appendix: Irradiation of a Cell Population with Heterogeneous Radio Sensitivity

The survival relation shown in Figure 1 is representative of that of a population of cells with heterogeneous radio sensitivity consisting of a mixture of subpopulations, each with homogeneous radio sensitivity expressed in its own distinct pair of values of the linear quadratic survival constants α and β. Figure 2 shows the survival experiment of Figure 1 transformed to a plot of $-\ln S/D$ versus D. On this plot the linear quadratic relation becomes a linear relation.

In the low dose range the survival relation of the population as a whole approaches the linear quadratic relation represented by line A. Let $\langle\alpha_i\rangle$ be the cell number weighted average of the diverse values of α, $\langle\beta_i\rangle$ be the cell number weighted average of the diverse values of β and $\langle\delta\alpha^2_i\rangle$ be the variance of the distribution of the values of α in the irradiated population. Let α be the intercept of line A at zero doses and β be its slope. It has been shown [5,73] that,

(A1) $\alpha = \langle\alpha_i\rangle$ and $\beta = \langle\beta_i\rangle - \frac{1}{2}\langle\delta\alpha_i^2\rangle$

These values of α and β are the survival constants that govern the survival of the population, as a whole, in the low dose range where the survival conforms to the linear quadratic relation of line A.

The linear quadratic relation represented by line B is approached as dose increases toward infinity. The slope of line B is the lowest value of beta in the population, called β_H. The intercept at zero dose of line B is the lowest value of alpha found among those cells with the lowest value of beta [5]. It is called α_H. The value β_H is often zero or near zero. The values of α_H and β_H are the survival constants that govern the linear quadratic survival of the population, as a whole, as dose increases toward infinity. In the intermediate range of dose where the curve of Figure 2 is concave downward the survival of the population as a whole is not linear quadratic.

Figure 4 shows a schematic representation of an isoeffect study like that to which equation 8 refers that would be found for a heterogeneous population of cells like that portrayed in Figure 1 and 2. The alpha beta ratio that governs the survival response for the low dose linear quadratic range is the ratio of the intercept at zero dose of line A to its slope. The alpha beta ratio that governs survival response for the high dose linear quadratic survival range is the ratio of the intercept at zero dose of line B to its slope. In the intermediate dose range the effective alpha beta ratio must be estimated from an interpolated average of that for low and high dose ranges. The zero dose intercept of line B divided by that of line A is the ratio α_H/α. The slope of line B divided by that of line A is the ratio β_H/β. The determination of the $1/D^*$ dependence on fractional dose from experiments with animals or from accumulated clinical experience of the occurrence of various organ injuries often defines only a limited segment of the curve of Figure 4.

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