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# Mechanistic Models

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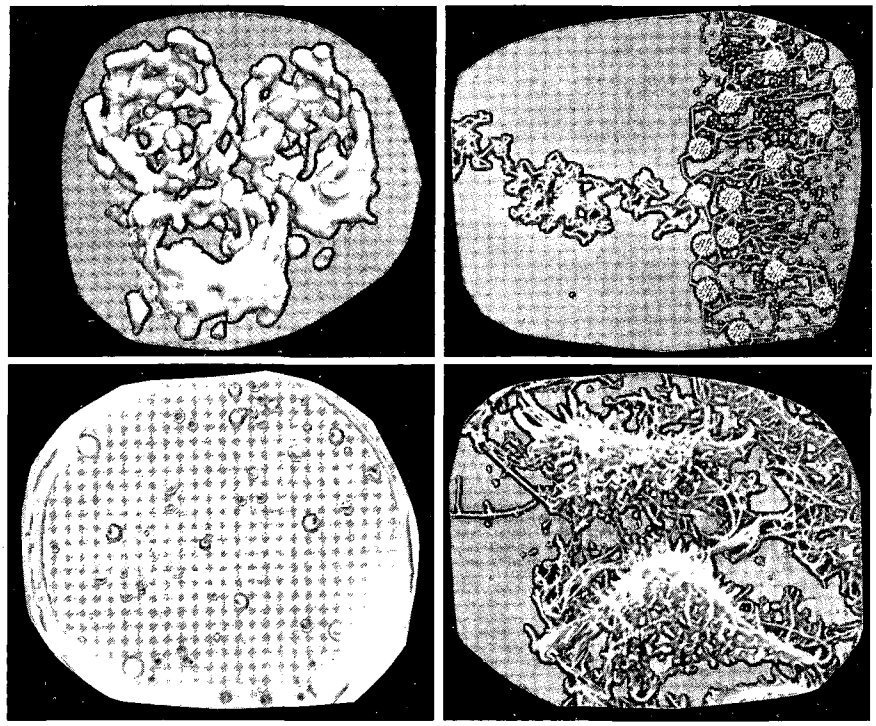



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# MECHANISTIC MODELS

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## Abstract

Several models and theories are reviewed that incoporate the idea of radiation-induced lesions (repairable and/or irreparable) that can be related to molecular lesions in the DNA molecule. Usually the DNA double-strand or chromatin break is suggested as the critical lesion. In the models, the shoulder on the low-LET survival curve is hypothesized as being due to one (or more) of the following three mechanisms: (1) "interaction" of lesions produced by statistically independent particle tracks, (2) nonlinear (i.e., linear-quadratic) increase in the yield of initial lesions, and (3) saturation of repair processes at high dose. Comparisons are made between the various approaches. Several significant advances in model development are discussed; in particular, a description of the matrix formulation of the Markov versions of the RMR and LPL models is given. The more advanced theories have incorporated statistical fluctuations in various aspects of the energy-loss and lesion-formation process. An important direction is the inclusion of physical and chemical processes into the formulations by incorporating relevant track structure theory (Monte Carlo track simulations) and chemical reactions of radiation-induced radicals. At the biological end, identification of repair genes and how they operate as well as a better understanding of how DNA misjoinings lead to lethal chromosome aberrations are needed for appropriate inclusion into the theories. More effort is necessary to model the complex end point of radiation-induced carcinogenesis.

## Introduction

There are many effects that ionizing radiation can have at the subcellular/molecular level. Those often mentioned are double- and single-strand breaks in the DNA, DNA base damage and DNA-protein crosslinks. Most of the theories/models that have been developed, however, have chosen the double- (and/or single-) DNA-strand break as the most likely lesion leading to reproductive cell death and other end points of interest such as mutation and cell transformation. Perhaps because of the large amount of experimental data available using *reproductive cell death* as an end point under many different physiological conditions and with many different cell types, the majority of models developed have chosen this end point as their focus.

It is important to recognize that this end point is crucial not only in fields such as radiation oncology where selective cell killing is the goal, but also in the field of radiation risk assessment where damaged but surviving cells are critical. Cell killing by radiation will modify the number of cells "at risk" for a transformation event and must be a part of any complete theory of radiation carcinogenesis. This is particularly true in the case of high-LET radiation carcinogenesis where the probability is not negligible that one traversal of a high-LET particle through the nucleus of a cell will kill it. For this reason, and because there are several relatively recent new ideas and developments involving this end point, the present review will emphasize the end point of cell reproductive death.

A good groundwork has been laid by the previous paper (Braby). The background has been covered and many different approaches have been discussed. It should be noted that although the present paper, as the title suggests, deals with mechanisms, the division between mechanistic and phenomenological models is tenuous at best and several of the approaches discussed here have phenomenological aspects. We will focus on different ideas behind the shape of the dose-response curve (survival curve) as suggested by mechanisms based on effects to the DNA molecule.

Such mechanisms can be divided into three general types: those involving "*interaction of lesions*," those requiring a *nonlinear induction of lesions*, and those involving *saturation of repair processes*.

Lesion Interaction Formulations

All interaction formulations have as a basis the idea that lesions or "sublesions" (i.e., sites of damage) can interact with other lesions to produce a lesion that ultimately leads to cell death. Such ideas can be traced originally to the ideas and quantitative description put forth by Lea and Catcheside (1942) of pieces of chromosomes misjoining to produce chromosomal aberrations.

Theory of Dual Radiation Action (Kellerer and Rossi, 1972, 1978).

This formulation arose out of a recognition (1) that it might well be important to include the stochastics of the energy-loss process in a theoretical treatment of radiation effects and (2) that the RBE of neutrons appeared to vary as the reciprocal of the square-root of the neutron dose for many different end points.

The basic hypotheses are:

1. Radiation effects in autonomous cells (i.e., cells responding independently of nearby cells also receiving radiation) and in particular, reproductive cell death, are due to lesions whose production is proportional to the square of the specific energy, z, deposited in the radiosensitive volume, called here the radiosensitive matrix.

2. These lesions are caused by pairwise interaction of molecular products termed "sublesions", whose production is proportional to z.

The specific energy, z, is a random variable and so the effect (or mean number of lesions) over a population of cells is written

$$\bar{\varepsilon} = k\,\overline{z^2} \tag{1}$$

It can be shown (Kellerer and Rossi, 1972) that

$$\overline{z^2} = \overline{z_{1D}}\,D + D^2 \tag{2}$$

where $\overline{z_{1D}}$ is the dose-mean specific energy for a single event. In the 1972 paper, it is called the "energy average" of the event size. The expression for the mean number of lesions becomes

$$\bar{\varepsilon} = k\,(\overline{z_{1D}}\,D + D^2) \tag{3}$$

This form of the model was used to permit the application of microdosimetric data, which provide values of $\overline{z_{1D}}$ experimentally for microscopically defined volumes of different sizes. With the assumption that $\bar{\varepsilon}$ was a measure of the mean number of lethal lesions in a cell, the survival was written as the zero class of a Poisson distribution, i.e., the probability that the cell has no lesions,

$$S = e^{-\bar{\varepsilon}} \tag{4}$$

Comparison with experimental data showed good agreement, if the critical volume had a characteristic size on the order of one or a few micrometers. This interpretation implies a site size and a constant interaction probability of sublesions produced inside the site.

The more generalized version (Kellerer and Rossi, 1978) addressed the question of a probability of sublesion interaction dependent on the separation of the sublesions. The formula for $\bar{\varepsilon}$ becomes

$$\bar{\varepsilon} = k\,(\xi D + D^2) \tag{5}$$

$$\text{where } \xi = \int_0^\infty \frac{s(x)\; g(x)\; t(x)\; dx}{4\pi\rho x^2} \Big/ \int_0^\infty s(x)\; g(x)\; t(x)\; dx \tag{6}$$

Here s(x)/V is the point-pair distribution of distances between points inside the sensitive matrix of volume V where energy transfers can result in sublesions, g(x) is the probability of interaction of two sublesions separated by a distance x, and t(x), the proximity function, is the point-pair distribution of energy-transfer points in a single event (i.e., a passage of an ionizing particle) weighted by the energy transferred. The quantity $\rho$ is the density of the sensitive matrix.

As pointed out by Kellerer and Rossi (1978), two single-strand breaks caused by independent electron tracks cannot be the sublesions leading to the lesion of a double-strand break relevant for cell killing, because at the doses experimentally found to cause the shoulder on the survival curve, the probability is negligible for energy transfers from two statistically independent tracks each to cause a single-strand break on opposite strands of the DNA molecule close enough to produce a double-strand break. More to the point would be two double-strand breaks representing, say, a chromatin break, interacting to produce a lethal chromosome aberration.

Recently, Brenner (1990) has carried the idea a step farther by explicitly assuming that the sublesions are double-strand breaks and the resulting lesions are exchange-type chromosomal aberrations. Also, the survival equation $S = e^{-k\bar{n}}$ with $\bar{n}$ being the mean yield of lesions is only true at low dose and LET, when $e^{-k\bar{n}}$ can be approximated by $1 - k\bar{n}$.

Therefore, he considered a cell-by-cell approach to be more appropriate. He simulated passage of radiation tracks through individual cells and has followed the production and interaction of double-strand breaks in time within each cell. Cell viability based on the number of exchange-type aberrations found in the cell was determined at an "appropriate" time. He used $10^4$ sec as the time of assay for viability (this is five times his assumed characteristic time for double-strand break repair). Another assumption in this approach is that one in 1500 ionizations along the track produces a double-strand break. The double-strand breaks were allowed to diffuse stochastically and interactions were scored if two were found within the "encounter radius", a, assumed to be 0.5 nm. It was first checked whether either of the two breaks had repaired via a process with characteristic repair time of 2000 sec. The probability for reaction was taken as $p(x,t) = \frac{a}{x}\,\mathrm{erfc}\,[(x-a)/\sqrt{4Dt}]$ where x is the original separation distance and Dt was taken as $12 \times 10^4$ nm$^2$. This gave a reasonable fit to the g(x) function of equation (6) as deduced from experiment. Of the exchange-type aberrations left at $10^4$ sec, one out of two was assumed to be lethal based on symmetry arguments. Good agreement between calculated and experimental survival curves was obtained for V-79 cells exposed to X-rays and ions with LET's from 20 to 170 keV/μm.

Repair-Misrepair Model (Tobias *et al.*, 1980)

Initial ("uncommitted") lesions are formed which can subsequently either interact pairwise to form a lethal lesion (quadratic misrepair) or misrepair on their own (linear misrepair) or repair correctly (linear eurepair).

It is convenient (although not essential) to assume that the initial uncommitted lesions are formed linearly with absorbed dose:

$$U_o = \alpha D \tag{7}$$

The kinetic differential equation describing the time-rate of change of the U lesions is

$$\frac{dU}{dt} = -\lambda U(t) - \kappa U^2(t) \tag{8}$$

where the linear term can be divided into two terms, $\lambda\phi\ U(t)$ and $\lambda(1-\phi)\ U(t)$, the eurepair and misrepair terms, respectively. The parameter $\phi$ gives the fraction of linear repair that is correct.

The solution to this equation is

$$U(t) = U_o\, e^{-\lambda t} \Big/ [1 + \frac{U_o}{\varepsilon}(1 - e^{-\lambda t})] \tag{9}$$

with initial condition $U(0) = U_o$ and where $\varepsilon = \lambda/\kappa$.

Lethal lesions are defined as the sum of the "uncommitted" plus linearly misrepaired plus quadratically misrepaired lesions at time t:

$$N_{\text{lethal}}(t) = U(t) + (1 - \phi)\lambda \int_o^t U(t')\ dt' + \kappa \int_o^t U^2\ (t')\ dt' \tag{10}$$

If $N_{lethal}$ is assumed to be the mean number of lethal lesions per cell of a Poisson distribution, the survival expression can be written:

$$S(t) = e^{-N_{\text{lethal}}(t)} \tag{11}$$

Making the appropriate substitutions, this yields

$$S(t) = e^{-\alpha D} [\ 1 + \frac{\alpha D}{\varepsilon}(1 - e^{-\lambda t})\ ]^{\varepsilon\phi} \tag{12}$$

This is the main result of the model. A subsequent change in parameter designation ($\delta$ replacing $\alpha$ and $\mu$ replacing $\varepsilon\phi$) yields

$$S(t) = e^{-\delta D} [\ 1 + \frac{\delta\phi D}{\mu}(1 - e^{-\lambda t})\ ]^{\mu} \tag{13}$$

The mechanistic interpretation of this model is that the initial uncommitted lesions are double-strand or chromatin breaks. Quadratic misrepair is due to separate broken strands rejoining ("interacting"), while linear misrepair is due to a single double-strand break misjoining in a way incompatible with cell survivability. The initial slope on the survival curve, assuming long repair times, is due to the infidelity of the linear repair process.

The space and time dependence of lesion formation (i.e., LET and dose-rate effects) has also been addressed (Tobias *et al*, 1980).

Recently, Albright (1989) has formulated lesion repair and misrepair in the RMR model as a Markov process, a discrete sequence of repair steps occurring at random times. This allows dropping the approximations of (1) neglecting the effect of statistical fluctuations in the repair process and (2) assuming the final lethal lesion distribution among cells to be Poisson.

Lethal Potentially Lethal Theory (Curtis, 1986, 1988)

This approach builds on the TDRA and RMR formulations and was originally presented as a unified repair theory (Curtis, 1983) joining several of the ideas from these approaches, plus the irreparable-repairable lesion concept found in the cybernetic model (Kappos and Pohlit, 1972).

The hypotheses on which the theory is based are:

1. Two broad classes of lesions are produced by ionizing radiation: irreparable and repairable. They are distinguishable by the amount of energy that must be deposited locally in order to produce them.

2. Irreparable (or "lethal") lesions are formed linearly with increasing absorbed dose with proportionality constant $\eta_L$.

3. Repairable (or "potentially lethal") lesions may be separated into at least two categories. Each is formed linearly with increasing absorbed dose, with proportionality constants $\eta_{PL}$ and $\eta_{PL}'$. They are distinguishable by their different repair rates. The rapidly repairing lesions, repairing with rate $\varepsilon_{PL}'$ (~ 3-6/hr), are not usually expressed as they are normally repaired with high fidelity and so are not experimentally measured in most experiments. The slowly repairing

lesions, repairing with rate $\varepsilon_{PL}$ (~0.5 to 1.0/hr), can be "fixed", i.e., made lethal, at various points throughout the cell cycle, or can "interact" with each other to form an irreparable (lethal) lesion, with rate $2\varepsilon_{2PL}$ (~ 0.05-0.15/hr). The latter process is called *binary misrepair*.

The molecular mechanisms are not specified. The values of the kinetic parameters $\varepsilon_{PL}$ and $\varepsilon_{PL}$, however, are consistent with the repair rates of slowly and rapidly repairing components of double-strand breaks, respectively.

The above hypotheses lead to the following differential equations:

1. During irradiation

$$\frac{dn_{PL}(t)}{dt} = \eta_{PL}\dot{D} - \varepsilon_{PL}\, n_{PL}(t) - \varepsilon_{2PL}\, n_{PL}^2\,(t) \qquad (14)$$

$$\frac{dn_{L}(t)}{dt} = \eta_{L}\dot{D} + \varepsilon_{2PL}\, n_{PL}^2\,(t) \qquad (15)$$

Here $\dot{D}$ is the dose-rate.

2. After irradiation

$$\frac{dn_{PL}(t)}{dt} = -\varepsilon_{PL}\, n_{PL}(t) - \varepsilon_{2PL}\, n_{PL}{}^2\,(t) \qquad (16)$$

$$\frac{dn_{L}(t)}{dt} = \varepsilon_{2PL}\, n_{PL}{}^2\,(t) \qquad (17)$$

If we assume the "high dose-rate approximation," i.e., repair occurring during the irradiation can be neglected, we have just equations (16) and (17) with initial conditions: $n_{PL}(0) = \eta_{PL}D$ and $n_{L}(0) = \eta_{L}D$.

The solution of these equations is straightforward (Curtis, 1988). With the assumption that the lesions are distributed among cells in a Poisson distribution and that the number of lethal lesions is the sum of the initially lethal and potentially lethal lesions at time, $t_r$, the survival can be written as the zeroeth class of the Poisson distribution, i.e., the probability that no lethal lesions exist in the cell:

$$S(t_r) = e^{-n_{PL}(t_r) - n_L(t_r)}$$

$$= e^{-(\eta_L + \eta_{PL})D} \left[1 + \frac{\eta_{PL} D}{\varepsilon}(1 - e^{-\varepsilon_{PL} t_r})\right]^{\varepsilon} \quad (18)$$

where $\varepsilon = \varepsilon_{PL}/\varepsilon_{2PL}$.

It can easily be shown (Curtis, 1986) that at low dose, the above equation approximates a linear-quadratic expression:

$$S = e^{-\alpha D - \beta D^2} \quad (19)$$

where

$$\alpha = \eta_L + \eta_{PL}\, e^{-\varepsilon_{PL} t_r} \quad (20)$$

$$\beta = \frac{\eta_{PL}^2}{2\varepsilon}(1 - e^{-\varepsilon_{PL} t_r})^2 \quad (21)$$

and, for low dose-rate and long repair time, $t_r$,

$$\beta = \frac{\eta_{PL}^2}{2\varepsilon} \cdot \frac{2}{(\varepsilon_{PL} T)^2}(\varepsilon_{PL} T + e^{-\varepsilon_{PL} T} - 1) \quad (22)$$

where T is the irradiation time. This is the "dose protraction" factor found in several of the other approaches.

A recent development of the LPL theory has included a Markov formulation (Curtis, 1988) for the high dose-rate version patterned after that obtained for the RMR Markov formulation (Albright, 1989). The conclusion of the reformulation is that for parameter values relevant to experimental survival curves, the error in assuming the Poisson rather than the Markov formulation leads to a 30% decrease in survival at 10 Gy absorbed dose. Such a difference would be very difficult to observe experimentally.

Some of the explicit predictions of this formulation for stationary-phase cells are:

1. For low dose-rates, the survival curve will have a constant slope and the shape will be independent of dose-rate.

2. For high dose-rates, the survival curve will be independent of dose-rate.

3. The *initial slope* of the delayed plating curve at high dose-rate will equal the slope of the low dose-rate curve (and be equal to $\eta_L$).

4. The initial slope of the survival curve of a proliferating cell population is dependent on the amont of time available for repair.

5. The slope of the survival curve will approach a constant ($\eta_L + \eta_{PL}$) as the dose increases.

6. Repair of sublethal damage and repair of the slow component of potentially lethal damage are different manifestations of the repair of the same lesions.

7. At low LETs, potentially lethal lesions dominate; at high LETs, lethal lesions dominate.

Nonlinearity of Initial Lesion Yield

The following two approaches assume that the shoulder on the survival curve is the reflection of the nonlinear yield of DNA double-strand breaks.

Critical DNA Target Size Model (Radford *et al.*, 1988)

In this model, it is hypothesized that DNA double-strand breaks are the critical lesions and that the dose response is nonlinear due to the action of a saturable chemical repair process. Only double-strand breaks occurring within "critical targets" are important, but these initiate recombination events with *undamaged sequences*, which lead to chromosomal aberrations. The subsequent loss of acentric fragments at mitosis prevents continuity of the genome and leads to cell death by inducing structural changes in the chromatin. Radford sites his own data on the yield of double-strand breaks (Radford, 1985, 1986a, 1986b) to support his contention that the yield is nonlinear at doses where a shoulder in the survival curve is seen.

One interesting assumption is that there are a number of critical targets of length $X_C$ along the DNA molecule that must remain intact for the cell to survive. This arises from the suggestion coming from experimental data that the number of lethal events is directly proportional to the number of DNA double-strand breaks per unit length of DNA. One

problem with this assumption is that $X_c$ appears to be dependent on the radiation type.

The targets are assumed to be sites of chromosome instability: the constitutive or common fragile sites (*c-fra*), and the protooncogenes occurring in "light G-bands" of stained chromosomes. In particular, the *c-abl, bcl-1, bcl-2, c-myc, and blym-1* genes are identified as being highly susceptible to radiation-induced breakage.

Since the non-linearity in this model occurs in the DNA double-strand break yield, the molecular mechanism for aberration production is presumed to be the damaged site initiating a recombination event with *undamaged* sequences. Asymmetrical exchanges would lead to cell death while symmetrical exchanges could lead to transformation. Double-strand breaks occurring in nontarget sequences are assumed to be repaired by a ligation-type mechanism and are not normally lethal to the cell. It is concluded that the repair of the vast majority of radiation-induced DNA double-strand breaks is irrelevant to an understanding of cell killing in normal mammalian cells.

Finally, it is hypothesized that cell death is due to the presence of chromosomal aberrations, particularly acentric fragments. The suggestion is that, in the interphase mammalian nucleus, chromosomal DNA is continuous, i.e., the chromosomes are joined together, and the existence of a fragment would prohibit the postulated proper interconnection because one chromosome would be missing a telomere. This produces long-range perturbation of chromatin structure and resultant changes in genetic activity which results in cell death. Such a mechanism is described as "karyotypic discontinuity."

Apparently, these ideas have not been quantified to the extent that a survival expression has been derived or deduced.

Molecular Theory (Chadwick and Leenhouts, 1981)

The unique aspect of the Molecular Theory is that single- and double-strand breaks in the DNA were hypothesized from the beginning as being the important molecular lesions. The survival curve shoulder is suggested to reflect the double-strand break yield curve as a function of absorbed dose.

The hypotheses are as follows:

1. Single-strand breaks are produced linearly with dose.

2. Double-strand breaks are produced when two single-strand breaks are produced in close proximity.

3. The production of lethal lesions is proportional to the double-strand break yield.

The result of the formulation is a yield of double-strand breaks having a linear component due to two closely produced single-strand breaks by a single charged particle, and a quadratic component due to two closely produced single-strand breaks by two statistically independent charged particles. The survival equation is written

$$S = e^{-p(\alpha D+\beta D^2)}. \tag{23}$$

As pointed out (Kellerer and Rossi, 1978), in the dose range below 10 Gy, microdosimetric arguments rule out the possibility that two single-strand breaks produced by two statistically independent tracks could produce the experimentally measured number of double-strand breaks. Also, in this range of doses, experimental evidence appears to favor a linear dependence of initial double-strand break yield with absorbed dose.

<u>DNA Fragment Loss and Unrepaired Double-Strand Breaks</u> (Ostashevsky, 1989)

This model assumes cell death is caused by the presence of an unrepaired double-strand break (including DNA fragments) at certain points in the cell cycle. It is concluded that each unrepaired double-strand break becomes a chromosomal aberration.

The survival expression is written:

$$S = [\, e^{-y} \sum_{k=0}^{\infty} (y^k/k!)\, \mu^{k_1-1}\, P(T)]^N \tag{24}$$

where N is the number of DNA molecules/nucleus, $y = D/D_{dsb}$ with $D_{dsb}$ = the mean dose for the induction of one double-strand break per molecule, $\mu$

is the probability that a DNA fragment will remain in the chromosome, $k_1 = k+1$ for linear DNA and $k_1 = k$ for circular DNA where k is the number of double-strand breaks per molecule, $P(T) = 1$ for $k = 0$, $P(T) = (1 - e^{-T})^k$ for $k > 0$ for repair processes where each double-strand break is repaired independently of other double-strand breaks, and $P(T) = 1 - e^{-T}$ for repair processes for which, if one break is repaired, all breaks on that molecule are repaired. $T = T_{rep}/\tau_{dsb}$ where $T_{rep}$ is the allowable repair time and $\tau_{dsb}$ is the mean time for double-strand break repair. In this expression, a sum is taken over k of the product of the (Poisson) probability of inducing k double-strand breaks times the probability that the fragment will remain in the chromosome times the probability that all k breaks in a molecule will be repaired by a time $T_{rep}$, and the whole expression raised to the $N^{th}$ power since there are N DNA molecules per cell nucleus.

A low dose-rate approximation is derived and shows exponential decrease with dose. Repair of potentially lethal damage is addressed and expressions are given for the (final) number of unrepaired double-strand breaks, and the (final) number of prematurely condensed chromatin fragments (PCC fragments). The interpretation is made that the final number of PCC fragments consists of two groups: those separated from DNA molecules soon after irradiation and those separated at the cutoff time for repair. All unrepaired double-strand breaks at the cutoff time become chromosome aberrations. A fraction yields DNA fragments, the rest interact to yield "misrepaired" chromosome aberrations.

The difference between radioresistant and radiosensitive cell lines lies in the relative amount of repair time available for double-strand break repair: long repair times relative to the characteristic double-strand break repair time leads to radioresistant cells and short repair time leads to a high level of "misrepair"-type chromosome aberrations and radiosensitive cells.

Repair Saturation

Many repair saturation models have been presented in various stages of development over the last decade and several were reviewed in the previous paper. Two models that relate to DNA damage and repair will be reviewed briefly here.

DNA Repair and Metabolic States (Wheeler, 1987)

In this approach, it is suggested that cell survival is related to the difference in rates of DNA lesion production and removal as mediated by the rates of metabolic processes required for maintaining cell integrity. It is specifically stated that the presence of residual unrepaired or misrepaired DNA lesions is not required to produce cell death. The probability of cell survival is written:

$$P(S) = 1 - \int_0^t [L(t) - R(t)]\, P_D(z)\, dt \qquad (25)$$

where $L(t)$ = number of DNA lesions produced per unit time
$R(t)$ = number of DNA lesions removed per unit time
$P_D(z)$ = probability of death/lesion/unit time given a specific metabolic state, z.

The idea of saturation of repair at high doses is introduced with the assumption that as the dose increases, the number of DNA lesions produced will eventually exceed the number of repair complexes available to remove the damage and the velocity of repair becomes a constant. In this situation, the half-time for damage removal will increase with dose. Experimental data have been presented that, it is claimed, support this idea, but analyses of data sets have not been quantitatively carried out explicitly using the above equation.

Damage Accumulation-Interaction (Reddy *et al.*, 1990)

The last model to be reviewed here is one in which the repair of damage relevant to cell survival is caused by a repair process that saturates at doses as low as 2 Gy. Interaction occurs among the accumulated (repairable) lesions during irradiation producing irreparable (lethal) lesions. The model is based on three hypotheses:

1. Lesions induced during irradiation either interact at the time of formation or do not interact at all and thus remain repairable provided the milieu does not alter DNA conformation.

2. Repair (in V79 cells) is not affected by postirradiation medium-dependent cell cycle progression or its delay.

3. Repair of repairable damage occurs in cells both with and without irreparable damage but is not detected by survival assays in cells with irreparable damage.

The unique aspect of this model appears to be the assumption that damage can interact only *during* the irradiation period. Experimental evidence in favor of this assumption is lacking at present.

The lesions suggested as being relevant for this model are breaks in chromatin and DNA which underlie the formation of chromosome aberrations. This model agrees with the preceding model (Wheeler, 1987) in that the repair process is unsaturated at low dose and is saturated at high dose (but in this model it is claimed that saturation occurs above 2 Gy for V79 cells irradiated at 2.5 Gy/minute dose-rate).

Explicit survival equations for this model have evidently not been published.

Comparison of the Models/Theories

A comprehensive comparison of even the theories and models chosen for this review would be too lengthy for inclusion in this presentation. A concise comparison, however, of the mechanism(s) hypothesized for the production of the shoulder on the survival curve is informative. Table I presents the mechanisms responsible for the shoulder of a low LET survival curve as envisaged by the developers of the formalisms under discussion. We see that even though DNA is assumed to be the critical target in all formulations and most assume that double-strand breaks or chromatin breaks leading to chromosomal aberrations are the important molecular lesions involved, there is still room for a varied interpretation of the "real" reason for a shouldered survival curve.

One important task for the future is to provide strong experimental evidence for or against at least the three mechanisms mentioned in the table: interaction of lesions, nonlinear yield of initial lesions, and saturation of repair processes. This, of course, assumes that the "lesions" have been identified. At present, the experimental evidence in the dose range below 6 Gy appears to favor interaction of lesions, since it is well established that broken pieces of chromatin/DNA do "misjoin" to form chromosomal aberrations which are lethal to the cell. In this dose range, the evidence is considerably weaker that there is nonlinearity in the

TABLE I

CAUSE OF SHOULDER ON THE LOW LET SURVIVAL CURVE

| Theory/Model | "Interaction" by lesions produced from statistically independent tracks | Nonlinear yield of initial lesions | Saturation of repair processes | Other | Reference |
|---|---|---|---|---|---|
| TDRA | √ | | | | Keller & Rossi 1972, 1978 |
| RMR | √ | | | | Tobias *et al.*, 1980, Tobias, 1985 |
| LPL | √ | | | | Curtis, 1983, 1986, 1988 |
| Molecular Theory | | √ | | | Chadwick and Leenhouts, 1981 |
| Critical DNA Target Size | | √ | √ | | Radford *et al.*, 1988 |
| DNA Fragment Loss & Unrepaired DSB's | | | | [Dose-dependent probability of fragment loss from chromosomes] | Ostashevsky, 1989 |
| DNA Repair & Metabolic States | | | √ | | Wheeler, 1987 |
| Damage Accumulation Interaction | √ | | √ | | Reddy *et al.* 1990 |

production of chromatin/DNA double-strand breaks or that the repair processes are saturating. A definitive paper has just appeared (Blöcher, 1990) showing, for the neutral elution technique of determining double-strand breaks, the nonlinear (e.g., linear-quadratic) dose response of the fraction of eluted DNA (or the logarithm of the fraction retained on the filter) does *not necessarily* imply nonlinear *induction* of double-strand breaks with absorbed dose.

TABLE II

DIFFERENCES BETWEEN DRA, RMR & LPL MODELS

Cell Survival End Point

| Model | Self-Repair | Self-Misrepair | Binary Repair | Binary Misrepair | Fixation |
|---|---|---|---|---|---|
| DRA | √ | | | √ | |
| RMR | √ | √ | (√) | √ | √ |
| LPL | √ | | | √ | √ |

(√) = present, but has negligible effect on cell survival.

Because the DRA, RMR and LPL formulations all hypothesize the same mechanism for the shoulder on the survival curve, it is informative to show explicitly the differences in these approaches. This is presented in Table II. The sublesions envisaged in DRA cannot be lethal individually but need an interaction with another sublesion to become lethal. This is not necessary in either the RMR or LPL approaches. Fixation of individual (sub)lesions can take place.This indeed is assumed to occur in experiments in which repair inhibitors, such as *araA* or hypertonic solution, are added to the cell medium. The difference between the RMR and LPL formulations is mainly that one type of "uncommitted" lesion is postulated in the RMR approach and the initial slope on the survival curve is due to

the infidelity of the repair process that causes individual lesions to misrepair (self-misrepair). In the LPL approach, the initially lethal irrepairable lesions (requiring more local energy deposition for their formation) are created linearly with dose and cause the initial slope on the survival curve.

Present and Future Directions

Clearly, this is a very active field with many interesting directions. Already mentioned is the need for definitive evidence in the dose region below 6 Gy for or against interaction of (sub)lesions, nonlinearity in the production of (sub) lesions with absorbed dose, and saturation of processes that repair the (sub)lesions. Several other significant directions for biophysical modeling have been discussed recently (Hall *et al.*, 1988). Briefly, it was concluded that in order to develop a complete understanding of the effects of ionizing radiation on a population of proliferating and non-proliferating cells, attention must be paid to (1) the statistical nature of the energy loss process through the concepts of microdosimetry and track structure theory, (2) the fluctuations of lesion formation from cell to cell throughout the population and (3) the variation of radiosensitivity throughout the cell cycle when proliferating cells are being considered.

Particularly interesting is the suggestion made recently by a joint DOE/CEC working group to compare various models by applying each to the analysis of the same sets of experimental data of one or more of three specific end points (cell transformation of C3H 10T1/2 cells, chromosomal aberrations and mutations, and interaction of mixed high- and low-LET radiations in cell survival experiments). A workshop scheduled in 1991 will bring the analyses together for comparison and evaluation.

Exciting progress is being made in extending the RMR and LPL models to introduce low dose-rate into the Markov formulations (Sachs *et al.*, 1990). In fact, this approach is a very general and elegant way of incorporating fluctuations into different theoretical descriptions. Briefly, the approach introduces a matrix formulation in which each matrix element relates to a differential equation describing the rate of change of lesions in a single cell. For example, eqs(14-17) above are replaced by individual probabilistic differential equations for the rate of change of the probability $P_n(t)$ of *one cell* having a given number of potentially lethal lesions, n. We will refer to a cell with n potentially lethal lesions as being in the nth state.

The equations in matrix element notation are:

$$\frac{dP_n(t)}{dt} = \sum_k M_{nk} P_k(t) \qquad n,k = 0,1,... \qquad (26)$$

where n is the number of potentially lethal lesions in a cell at time, t, and k denotes the sources [i.e., states that are involved in the movement of lesions into (or, when k=n, out of) the state of n lesions].

The $M_{nk}$ are matrix elements that correspond to transitions from the $k^{th}$ state to the nth state or (when k=n) out of the $n^{th}$ state.

The form of the matrix **M** is

$$\mathbf{M} = D'/z_F\ \mathbf{R} + \mathbf{A} \qquad \text{during irradiation} \qquad (27)$$
$$\mathbf{M} = \mathbf{A} \qquad \text{after irradiation} \qquad (28)$$

Here D' is the dose-rate and $z_F$ is the average specific energy per event which relates the energy deposition (i.e., the dose) in the nucleus to the number of events.

The matrix **A** contains the transition rates which involve the repair and misrepair processes and are operative both during and after irradiation:

$$A_{nn} = -n\,\varepsilon_{PL} - n(n-1)\,\varepsilon_{2PL} \qquad \text{for } k = n \qquad (29)$$
$$A_{n,n+1} = (n+1)\,\varepsilon_{PL} \qquad \text{for } k = n+1 \qquad (30)$$

The biological interpretation of these equations is that the number of cells in the nth state is changing in two ways: first by cells moving out of the state by "correct" repair (n lesions can be repaired, which occurs at a rate $\varepsilon_{PL}$ per lesion per unit time) and by "incorrect" binary misrepair (there are 1/2 n(n -1) pairwise interactions that can take place which occur at a rate 2 $\varepsilon_{2PL}$ per lesion$^2$ per unit time), and, secondly, by cells moving into the state from the $(n+1)^{th}$ state with lesions being repaired with a rate $\varepsilon_{PL}$ per unit time.

The use of just the **A** matrix (**R** = 0) yields the results already reported in Curtis (1988) for the LPL theory and Albright (1989) for the Repair-Misrepair theory. This is valid for dose-rates large enough and exposure times small enough so that repair during the radiation time can be neglected.

For lower dose-rates and long exposure times, lesions are being produced by the radiation and repaired by the cell during the same time period, so both processes must be included in the matrix. Thus, the **R** matrix elements, which relate to the production of lesions by the radiation, are introduced:

$$R_{nn} = -\mu - \nu \qquad \text{where } \mu = \sum_k \mu_k \tag{31}$$

$$R_{n,n-k} = \mu_k \tag{32}$$

where $\mu_k$ is the probability of producing k potentially lethal lesions in one event, and $\nu$ is the probability of producing one or more lethal lesions in one event. This approach expresses the irradiation process as a series of events which, in turn, produce lesions. This appears to be a convenient and conceptually appropriate manner with which to describe the irradiation process, irrespective of the value of the dose-rate.

Written in matrix notation, where dP/dt on the left hand side is a one-row matrix and P on the right hand side is a one-column matrix:

$$\mathbf{dP}(t)/dt = \mathbf{M}\,\mathbf{P}(t). \tag{33}$$

The formal solution is

$$\mathbf{P}(t_r) = e^{\mathbf{M}\,t_r}\,\mathbf{P}(T) \tag{34}$$

where

$$\mathbf{P}(T) = e^{\mathbf{M}\,T}\,\mathbf{P}(0) \tag{35}$$

and where the matrix **M** is the appropriate one applying during the radiation [**M** is taken from eq.(27) for eq.(35)] or after the irradiation [**M** is taken from eq.(28) for eq.(34)].

The probability for a cell to survive is the probability of a cell having no potentially lethal or lethal lesions at $t_r$, the time available for repair after irradiation. It is given by:

$$S = P_0 ( t_r ) \tag{36}$$

where $t_r$ is the time post-irradiation after which no repair can take place and the fate of the cell has been determined, and subscript zero indicates no lesions are present.

The overall approach described here provides the capability for calculating statistical distributions of potentially lethal and lethal lesions in a cell population at various times after radiation. Such distributions may ultimately be used to compare theoretical predictions with experimental distributions of "candidate" lesions (e.g., chromatin breaks). Another advantage of this formulation is that various other hypotheses can be accommodated such as saturable repair, multi-target and multi-hit effects, certain aspects of the dual-radiation-action hypothesis, etc. Two additions of particular interest are cell-cycle effects and the LET-dependence of the probability of potentially lethal and lethal lesion formation per event.

Finally, end points such as transformation, mutation and tumorigenesis should be addressed within the framework of the statistical fluctuations discussed above. The concept that carcinogenesis does not arise from autonomous cells should be taken seriously. This idea confounds the development of conceptually simple theories, but, if true, cannot be neglected in our ultimate understanding of radiation-induced carcinogenesis.

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