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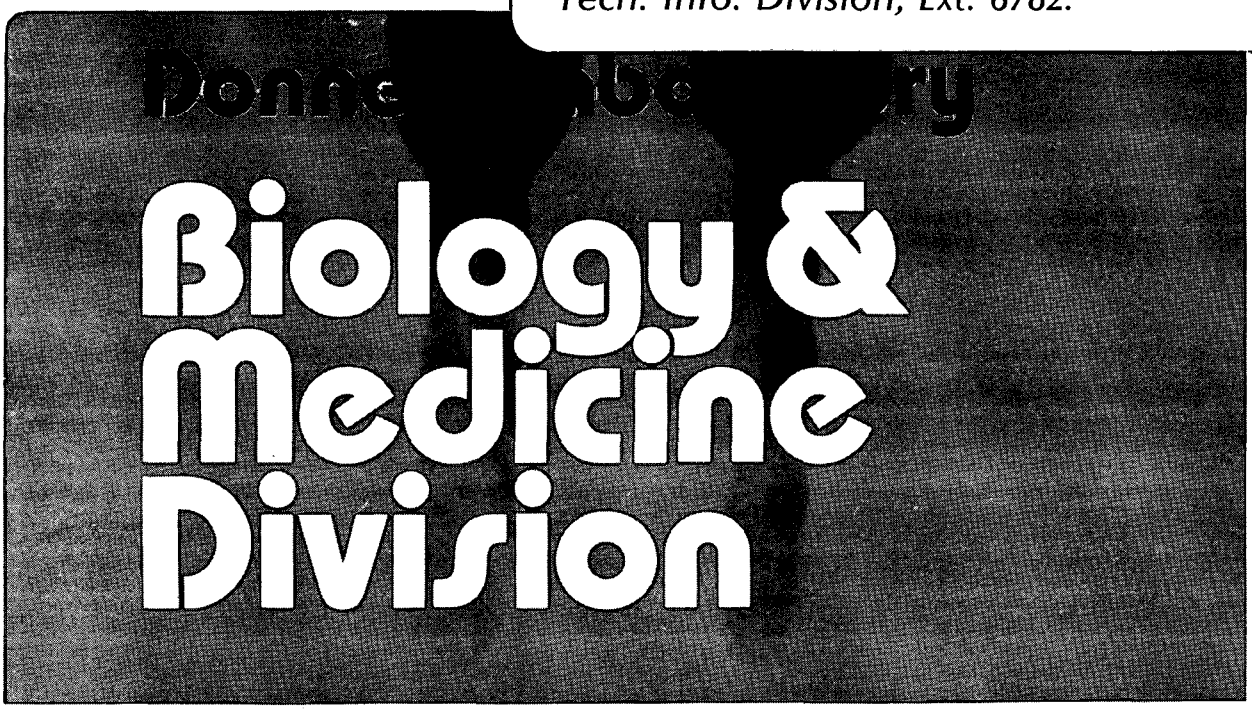

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IDEAS ON THE UNIFICATION OF RADIOBIOLOGICAL THEORIES

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ABSTRACT

A unified formulation of cell inactivation has been developed that incorporates major ideas of several theories (hypotheses) of how individual mammalian cells are inactivated by ionizing radiation. Elements from the repair-misrepair, lethal-potentially lethal, sublesion interaction, and track structure models are combined to produce a single set of mutually compatible hypotheses.

The basic elements of the unified model are:

1. Long-lived "sublesions or "potentially lethal" lesions (half-lives of many minutes) are formed by the radiation field. The lesions can interact over large distances (micrometers) during the enzymatic repair process to form lethal lesions via "misrepair" processes. These lesions can be identified with "intertrack interactions." They have a finite probability for being repaired correctly.
2. One charged particle track can form lesions, some of which (depending on ionization density) may be created in such close proximity that two or more can interact strongly (i.e., in short times) to form lethal lesions. The lesions, arising from single tracks, can be identified with "intratrack interactions."
3. The sublesions are produced by several closely spaced ionizations (perhaps as many as six to ten are needed for each sublesion within a distance of 3 nanometers). The important physical parameters to describe the radiation field become the fluence of particles and the mean free path for creation of such "clusters" of ionizations along the track.

The chemical environment within the cell nucleus will determine whether a number of ionizations in close proximity will become a "potentially lethal" lesion.

Several consequences arise from this formulation:

1. The most "fundamental" cellular survival data are obtained with stationary phase cell cultures using either very low dose rates, or "delayed plating" procedures to allow maximum repair. From the former, values for the irrepairable component of damage can be obtained. The delayed plating experiments (at conventional dose rates) yield the amount of long-range lesion interaction, which causes a shoulder on the survival curve. "Immediate plating" curves (even of stationary phase cultures) are more difficult to interpret because the fresh medium placed on the cells initiates the mitotic cycle and causes a "fixation" of damage either by interaction between a lesion and the chemical components of the medium, or at "fixation points" within the cell cycle.
2. At low LET, the rate of irreversible damage production (α) increases linearly with LET because it varies as const/($\overline{L}_T \cdot \lambda^2$), where $\overline{L}_T$ is the track average LET and λ is the mean free path along the track between "sublesions." To the extent that λ is inversely proportional to ionization density (hence, to LET), α will increase linearly with LET.
3. The limiting slope at high dose is a measure of the production rate (per unit dose) of the total number of lethal and "potentially lethal" lesions initially produced by the radiation.

Introduction

The ideas embodied in several of the more prominent models of radiation action are, at first glance, difficult to reconcile. The spatial extent of site sizes or lesion interaction distances implied by the dual theory of radiation action of Kellerer and Rossi (1972, 1978) is somewhat larger than characteristic dimensions of suggested target molecules such as the DNA double helix. The double strand break hypothesis of Chadwick and Leenhouts (1978) has had to be modified to include a larger dimension such as a diffusion distance of a reactive radical species. The hypothesis of two kinds of initial damage (irrepairable and repairable) (e.g., Pohlit and Heyder, 1981) appears, perhaps, inconsistent with the suggestion of one type of lesion initially formed by the radiation field (Tobias et al., 1980).

A description is presented here of a model that includes several of the ideas mentioned above. Thus, the resultant formulation can be described as a "unified" model in the sense that a single set of mutually compatible hypotheses are formulated that embrace a number of seemingly disparate ideas.

Major Hypotheses of the Model

The model, which we will call the LPL (lethal, potentially lethal) model, is based on the following major hypotheses:

1. Long-lived "potentially lethal" sublesions initially separated spatially from each other, with lifetimes of many minutes, are created in a cell nucleus by a (low LET) radiation field. These sublesions can interact during the enzymatic repair process even though they were created long distances apart (on the order of micrometers). The interactions cause lesions that result ultimately in loss of cell reproductive capacity ("lethal lesions"). This process obviously depends on the square of the lesion concentration. Such interactions can be called "misrepair" processes and can be identified with "quadratic misrepair" in the RMR formulation (Tobias et al., 1980). Sublesions can also be repaired correctly. The probabilities of correct repair and misrepair depend not at all on the initial proximity of one lesion to another, but only on their overall concentration.

2. Radiation-induced sublesions can also interact with each other to create a "lethal" lesion on a very short time scale (on the order of a second or less) if they are created simultaneously and very close together (for instance, by a single charged particle). The cell cannot respond to these sublesions individually; thus, as far as the cell is concerned, a "lethal" or irrepairable lesion is formed immediately. As a first approximation, we assume that sublesions formed within a separation distance x_o of each other along the track of a charged particle will form a lethal lesion, and no (immediate) lethal lesion will be formed if the separation distance is greater than x_o. The value of x_o will vary with the chemical composition of the environment within the cell; in many cases it is probably on the order of tens of nanometers.

3. The sublesions are assumed to be caused by groups or clusters of ionizations. Perhaps six to ten ionizations are necessary within a distance of 2 to 3 nanometers to produce a sublesion with high probability.

The sublesion interactions at long times can be identified with "inter-track" or "two-track" interactions (Neary, 1965) and "gamma kill" (Katz et al., 1971), because they predominate at low LET. The short range fast interactions can be identified with "intratrack" or "one-track" (Neary, 1965) processes and "ion kill" (Katz et al., 1971), because they occur along a single charged particle track and become more important at high LET.

Development of a Survival Curve Expression

With the above hypotheses plus the assumption that the numbers of potentially lethal and lethal lesions per cell follow Poisson statistics, we proceed to develop the survival expression.

Because this portion of the model is essentially that proposed by Pohlit, except for the misrepair term depending on the square of the concentration and the repair term depending linearly on the concentration, we use the notation and formalism developed by him (see Kappos and Pohlit 1972; Pohlit and Heyder, 1981). Figure 1 shows the basic structure of the model in its simplest form. Let $n_B(t)$ = the mean number of potentially lethal lesions per cell at time t, and $n_C(t)$ = the mean number of irrepairable (lethal) lesions per cell at time t. We now write the differential equations for the time rate of change of concentrations of lesions:

$$dn_B(t)/dt = -\epsilon_{BA}n_B(t) - \epsilon_{BC}n_B^2(t) \quad (1)$$

$$dn_C(t)/dt = \epsilon_{BC}n_B^2(t) \quad (2)$$

with the initial conditions:

$$n_B(0) = \eta_{AB}D \quad (3)$$

$$n_C(0) = \eta_{AC}D \quad (4)$$

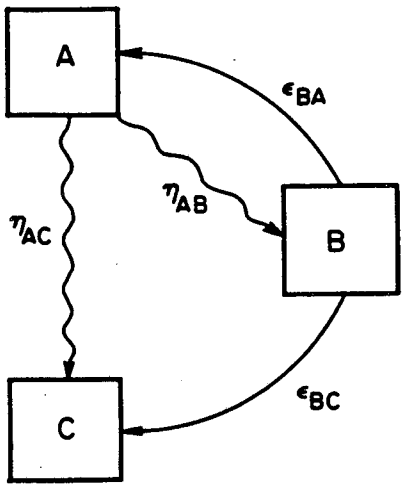

Figure 1. Basic schematic representation for the production, repair, and misrepair of radiation lesions. η_{AB} and η_{AC} denote the rates of production (per unit absorbed dose) of repairable and irrepairable damage, respectively. ε_{BA} and ε_{BC} denote the rates (per unit time) for enzymatic repair and misrepair, respectively.(XBL 829-4114)

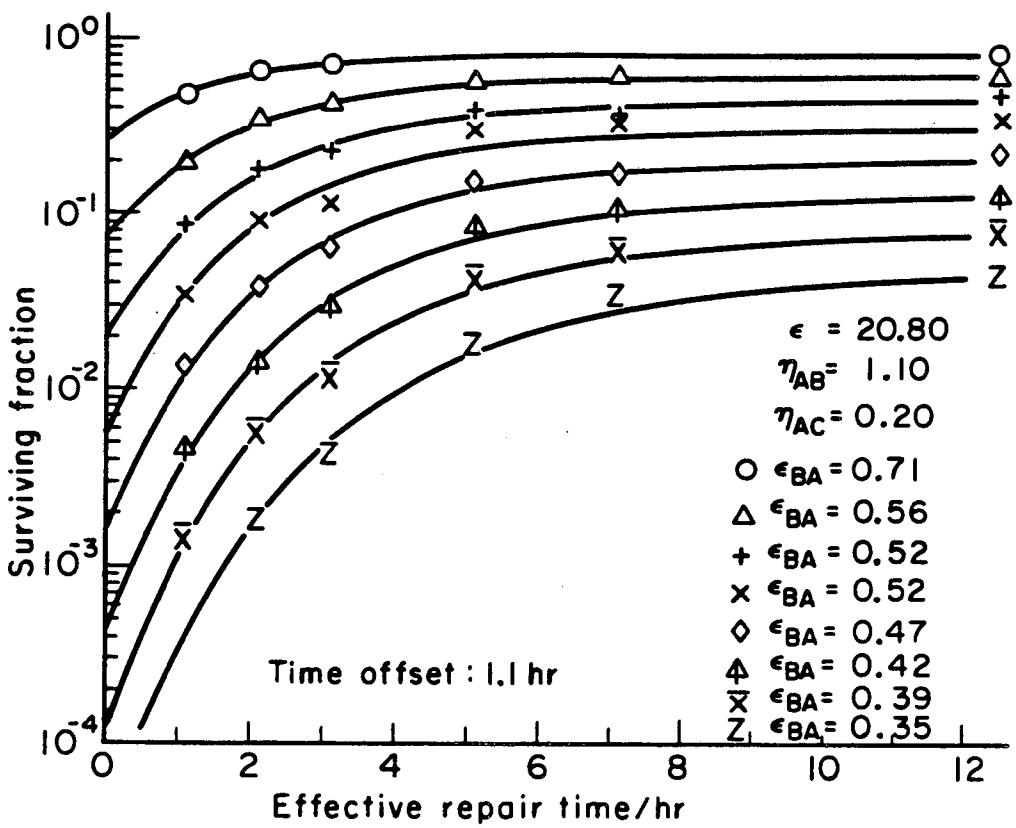

Figure 2. Comparison of the model calculations with experimental data of repair kinetics for stationary phase Ehrlich ascites tumor cells from Iliakis (1980) using β-araA to inhibit repair. The eight sets of data were obtained after doses of 1 through 8 Gy. The model parameters are defined in the text. (XBL 829-4113)

where η_{AB} and η_{AC} are the production rates per unit dose for the potentially lethal and the lethal lesions, respectively. ε_{BA} is the coefficient for correct repair and ε_{BC}, the coefficient for misrepair. The solutions to these equations can be written:

$$n_B(t) = \eta_{AB} D e^{-\varepsilon_{BA} t} \Big/ \left[1 + \frac{\eta_{AB} D}{\varepsilon}\left(1 - e^{-\varepsilon_{BA} t}\right)\right] \tag{5}$$

where $\varepsilon \equiv \varepsilon_{BA}/\varepsilon_{BC}$

$$n_C(t) = \varepsilon \left\{ \left[(1+\eta_{AB} D/\varepsilon)\eta_{AB} D/\varepsilon \cdot \left(1 - e^{-\varepsilon_{BA} t}\right)\right] \Big/ \left[1+\eta_{AB} D/\varepsilon \cdot \left(1 - e^{-\varepsilon_{BA} t}\right)\right] - \ln\left[1+\eta_{AB} D/\varepsilon \cdot \left(1 - e^{-\varepsilon_{BA} t}\right)\right] \right\} + \eta_{AC} D \tag{6}$$

In order to calculate the survival, we follow the method outlined in the RMR model (Tobias et al., 1980) and make the following assumptions:

1. All n_C lesions are lethal (misrepair).
2. All n_B lesions become lethal if the repair process is halted by some experimental treatment (fixation of damage).
3. At any time, $t \neq 0$, the distribution of lesions per cell can be considered a Poisson distribution.

The survival expression is simply:

$$S(t) = e^{-n_T(t)} \tag{7}$$

where $n_T(t)$ is the total mean number of lesions per cell at time, t, and equals:

$$n_T(t) = n_B(t) + n_C(t) \tag{8}$$

Combining eqs. (5) and (6) and substituting them in eq. (7), we obtain:

$$S(t) = e^{-(\eta_{AC}+\eta_{AB})D} \left[1+\eta_{AB} D/\varepsilon \cdot \left(1 - e^{-\varepsilon_{BA} t}\right)\right]^{\varepsilon} \tag{9}$$

The "delayed plating" expression is obtained when t is set equal to ∞, i.e., for maximum repair:

$$S(\infty) = e^{-(\eta_{AC}+\eta_{AB})D} \left[1+\eta_{AB} D/\varepsilon\right]^{\varepsilon} \tag{10}$$

At t=0, if measurements could be made, the survival curve would be exponential:

$$S(0) = e^{-(\eta_{AC}+\eta_{AB})D} \tag{11}$$

Unfortunately, "immediate plating" experiments are difficult to interpret because experiments indicate that some repair does occur in the fresh medium placed on the cells immediately after irradiation (Iliakis, 1981; Pohlit and Heyder, 1981).

Experiments at very low dose rates should yield exponential survival curves. In this case, the concentration of lesions remains low, the quadratic term in eq. (1) will remain small and can be neglected. All potentially lethal lesions will be correctly repaired and the resulting slope is a measure of η_{AC}, the production coefficient for irrepairable damage. Thus, the most "fundamental" curves are obtained from low dose rate and "delayed plating" experiments using stationary phase cell cultures.

As an example of the application of this model to a set of experimental data, Figure 2 shows the survival of stationary phase Ehrlich ascites tumor cells as a function of "effective repair time" before treatment with the DNA synthesis inhibitor, β-arabinofuranosyladenine (β-araA) (Iliakis, 1980). A graded series of doses was given from 1 Gy (top data set) to 8 Gy (bottom data set). The data just off scale to the right are for the "delayed plating" time (20 hrs.). Best fit curves were obtained by varying ε, η_{AB} and an "offset time" all independently. The values of ε_{BA} were obtained by varying each curve for a best fit. The "offset time" denotes that interval of time (in this case 1.1 hrs.) that was added to the true interval between irradiation and addition of the drug to allow for repair occurring during penetration of the drug into the cell nuclei and the initiation of the inhibition.

High LET Effects

To treat the variation of LET, it is convenient to consider the radiation as consisting of individual charged particle tracks. Thus, we can replace $\eta_{AC}D$, the mean number of initial irrepairable lesions, with $\bar{\sigma}_{AC} \cdot \Phi$ where Φ is the total fluence of charged particles and $\bar{\sigma}_{AC}$ is the average cross section per cell nucleus for immediate lethal lesion formation.

Noting that, from the definition of track averaged LET, $\bar{L}_T$, we have:

$$D = \bar{L}_T \; \Phi \tag{12}$$

and setting:

$$\eta_{AC} D = \bar{\sigma}_{AC} \Phi \tag{13}$$

we see immediately from eqs. (12 and 13):

$$\eta_{AC} = \bar{\sigma}_{AC} / \bar{L}_T \tag{14}$$

It will be more convenient as well as instructive from here on to consider the cross sections $\bar{\sigma}_{AC}$ and $\bar{\sigma}_{AB}$ for lesion formation instead of the parameters η_{AC} and η_{AB}.

The average cross section may be written:

$$\bar{\sigma}_{AC} = 1/\Phi \cdot \sum_{i=1}^{n} \bar{\sigma}_{AC,i} \Phi_i \tag{15}$$

$$\text{with } \Phi = \sum_{i=1}^{n} \Phi_i$$

Here the sum is over the different kinds of particles present (total = n). The average cross section for the $i^{\underline{th}}$ particle can be written:

$$\bar{\sigma}_{AC,i} = 1/\Phi_i \cdot \int \sigma_{AC,i}(L_i) \Phi_i(L_i) \, dL_i \tag{16}$$

or, it can be averaged over any suitable physical parameter, for example, β, the velocity relative to that of light:

$$\bar{\sigma}_{AC,i} = 1/\Phi_i \cdot \int \sigma_{AC,i}(\beta_i) \Phi_i(\beta_i) d\beta_i \tag{17}$$

Hypotheses for Developing the Cross Sections

We now make assumptions as to how irrepairable lesions and potentially lethal lesions are formed.

1. The irrepairable lesions are created by an interaction of two "sublesions" (perhaps, potentially lethal lesions) formed by a single charged particle track (including its delta rays).

2. The probability for this lethal interaction of lesions depends on the separation distance between them. As a first approximation, we assume that the probability is unity for separation distances along the track less than some value x_o, and zero for greater separation distances.

3. The sublesions are distributed along the track according to Poisson statistics.

4. The distance of the sublesions from the charged particle trajectory is small compared to the mean distance between them. That is, the sublesions can all be considered to lie along the trajectory.

5. A sublesion is created by a number of closely spaced ionizations. Although the numbers and distances are not known, an assumption based on reasonable sizes of target molecules is that perhaps six to ten ionizations might have to lie within a volume with characteristic size of 2 to 3 nanometers.

6. The radiosensitive material is distributed in a volume of cross section σ_o and mean chord length ℓ_o.

With the assumption of Poissonian statistics, we can proceed as follows: First, we define a mean number of sublesions per unit track length, $\overline{N_i}$, for the $i^{\underline{th}}$ particle; then: $1/\overline{N_i} = \lambda_{\ell,i}$ the mean free path for lesion production by the $i^{\underline{th}}$ particle. The probability for zero sublesions within x_o is $e^{-x_o/\lambda_{\ell,i}}$; and the probability for one sublesion within x_o is $(x_o/\lambda_{\ell,i})e^{-x_o/\lambda_{\ell,i}}$. Therefore, the probability for two or more sublesions within x_o is:

$$P_{\geq 2} = 1-e^{-x_o/\lambda_{\ell,i}} - x_o/\lambda_{\ell,i}\, e^{-x_o/\lambda_{\ell,i}} = 1 - e^{-x_o/\lambda_{\ell,i}}\,(1+x_o/\lambda_{\ell,i}) \qquad (18)$$

which is the probability for the formation of <u>irrepairable</u> <u>lethal</u> <u>lesions</u>.

Therefore:

$$\sigma_{AC,i} = \sigma_o \left[1-e^{-x_o/\lambda_{\ell,i}}\cdot(1+x_o/\lambda_{\ell,i})\right] \qquad (19)$$

Finally, if we assume that the sublesions defined for the lethal lesions above are the potentially lethal lesions, we need the probability that one and only one lesion lies in x_o, times the number of such distances, ℓ_o/x_o:

$$P_1 = \left(x_o/\lambda_{\ell,i}\right)\, e^{-x_o/\lambda_{\ell,i}}\, \left(\ell_o/x_o\right) = \left(\ell_o/\lambda_{\ell,i}\right)\, e^{-x_o/\lambda_{\ell,i}} \qquad (20)$$

Therefore:

$$\sigma_{AB,i} = \left(\sigma_o \ell_o/\lambda_{\ell,i}\right)\, e^{-x_o/\lambda_{\ell,i}} \qquad (21)$$

At low LET, the expressions simplify to:

$$\sigma_{AC,i} = \sigma_o x_o^2/2\lambda_{\ell,i}^2 \qquad (22)$$

and

$$\sigma_{AB,i} = \sigma_o \ell_o / \lambda_{\ell,i} \tag{23}$$

Therefore, if $1/\lambda_\ell = \overline{N}$ increases linearly as the LET increases, which may not be a bad approximation at low LET, σ_{AC} increases as LET^2, so η_{AC} will increase linearly with LET. Similarly, σ_{AB} increases linearly as LET and η_{AB} will remain constant. At high LET, σ_{AC} approaches the saturation value of σ_o; in contrast, σ_{AB} reaches a maximum and then decreases to zero.

Inserting reasonable values for the parameters in eq. (23) shows that σ_{AB} at low LET is unreasonably large. There is no reason to expect, however, that all the isolated clusters will result in potentially lethal lesions. For instance, it is possible that "fast" chemical restitution processes may decrease the number of potentially lethal lesions. Here is a natural place then to introduce into the model the chemistry we know is going on. It is, in fact, to be expected that chemical reactions play an important role in determining the fate of the initial lesions. Finally, in addition to σ_{AB} being dependent on chemistry through fast restoration processes, we anticipate that x_o is also a strong function of the chemical environment.

In conclusion, an attempt has been made to bring together in a unified picture several of the ideas contained in various models currently being discussed. A set of mutually compatible hypotheses has been developed that incorporate ideas of repair, lesion formation, low dose rate effects and LET effects. Finally, it is indicated where the chemistry may play a role in modifying the number of initial lesions with which the the cell must ultimately deal.

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