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LETHAL AND POTENTIALLY LETHAL LESIONS INDUCED BY RADIATION - A UNIFIED REPAIR MODEL

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ABSTRACT

A model of radiation action is described which unifies several of the major existing concepts which have been applied to cell killing. Called the Lethal and Potentially Lethal (LPL) Model, it combines the ideas of lesion interaction, irrepairable lesions caused by single tracks, linear lesion fixation, lesion repair via first order kinetics and binary misrepair. Two different kinds of lesions are hypothesized: irrepairable (lethal) and repairable (potentially lethal) lesions. They are tentatively being identified with DNA double strand breaks of different severity. Two processes compete for depletion of the potentially lethal lesions: correct repair following first order kinetics and misrepair following second order kinetics. Fixation of these lesions can also occur. The model applies presently only to plateau (stationary) phase cells. Radiobiological phenomena described include effects of low dose rate, high LET, and repair kinetics as measured with repair inhibitors such as hypertonic solution and β-araA. One consequence of the model is that repair of sublethal damage and the slow component of potentially lethal damage are two manifestations of the same repair process. Hypertonic treatment fixes a completely new class of lesions which normally repair correctly. The "dose rate factor" occurring in several linear-quadratic formulations is shown to emerge when appropriate low-dose and long-repair-time approximations are made.

Key words: cell survival models, ionizing radiation, repair kinetics, plateau phase, mammalian cells, low-dose rate, high LET, potentially lethal damage, sublethal damage, DNA double strand breaks.

INTRODUCTION

One underlying motivation for formulating models of radiation action is to develop the means to describe quantitatively the results of radiobiological experiments with parameters that reflect the mechanisms of action at the physical, chemical and/or biological levels. Thus, it would not be surprising that the parameters of a successful model should depend on physical, chemical and biological variables in the environment. In an entity as complex as a living cell, it is not unreasonable to expect that there may be several, perhaps many, levels of models to be developed which will ultimately provide the quantitative expression of our understanding of the processes leading to a particular end point.

In the present formulation, we will restrict ourselves to the end point of cell survival (more accurately, retention of clonogenic capacity). In addition, we will consider only quiescent or resting cells. Cells moving through the cell cycle present problems that within the context of the present model have not been solved.

The present formulation, which we call the lethal, potentially lethal (LPL) model, has developed from ideas embodied in several of the more prominent theories and models in the recent literature. In this sense, the LPL model can be considered a unified model. The most pervasive underlying idea, that of competition between lesion repair and misrepair occuring long after the initial energy deposition, arises from the Repair-Misrepair (RMR) formulation (1). As will be seen, it also includes such ideas as the interaction of lesions (The Theory of Dual Radiation Action (2,3) and the RMR model (1)), intra- and intertrack contributions (4, 5), and repairable and irrepairable damage (6-8). In addition, in identifying the potentially lethal lesion conceptually with a double strand break in DNA, we attempt to tie the model to a well-known

molecular lesion, as has also been done by Chadwick and Leenhouts (9) in their "molecular theory". This identification is not absolutely necessary and is only done at present because of the rough equivalence between the mean repair times observed for cellular repair and the repair of double strand breaks (~90-120 minutes).

Repair of damage is treated explicitly. One consequence of the formulation is that the amount of time available for repair after irradiation affects the initial slope of the survival curve as well as the extent of the shoulder region. The overall shape of a survival curve obtained at high dose rates (i.e., rates for which curve shape is independent of dose rate) has, in the present model, the following characteristics[1]:

1) There is an initial non-zero slope.
2) There is a region of this curve at low dose that can be approximated by a linear-quadratic function in the absorbed dose.
3) At high doses, the slope of the survival curve approaches a constant which is a measure of the total number of initial biological lesions created by the radiation and relevant to survival.

Classification of Radiolesions

The following picture is assumed to describe in qualitative terms the progression of events occurring after irradiation of a population of living cells. The radiation itself causes many different kinds of physical, chemical and biological products or lesions. We assume that there is an evolution of these lesions from one to another with time. Very short-lived physical events (excitation and ionizations lasting less than 10^{-15} second) produce water radicals and other chemical lesions (lasting less than a second) which can diffuse through and/or react with molecules in the cell, thus creating

biomolecular lesions in the organic material within the cell and these can last a relatively long time (minutes or hours) in a quiescent cell. The entire process is extremely complex. Thus, the point of view is taken in the present formulation that important events occurring on the physical and chemical time scales create longer lived products which we will call biological lesions and which can interact or repair over a long time interval. The simplifying assumption is made [as in the RMR model (1)] that, over the range of dose rate considered here, physical or chemical lesions from statistically independent charged particle tracks do not interact. Thus, only lesions from single charged particle tracks (and their associated delta rays) need be considered in the physical and chemical time domains (less than one second). These lesions in turn create the longer lived biological lesions, some of which can repair enzymatically or interact with other lesions. In this way, the model can be broken into two distinct parts: one part dealing with the repair and/or interaction of the biological lesions and the other dealing with the creation of the biological lesions through the time evolution of first the physical and then the chemical lesions. The three time domains are shown in Figure 1 and the general categories of important lesions are indicated in each time frame. We emphasize that the explicit assumption is made here that the only interactions between lesions formed by different charged particle tracks are by the biological lesions in the long time frame.

Repair and Interaction Kinetics of the Biological Lesions

The major assumptions of the biological or long time scale portion of the model are as follows:

1) Two different kinds of biological lesions relevant to cell killing are created in the radiosensitive material within a cell during irradiation:

"lethal" and "potentially lethal" lesions. Lethal lesions are irrepairable and lead to the death of the cell or its progeny. Potentially lethal lesions are capable of being repaired and are correctly repaired at an average rate constant ε_{PL} per unit time. These lesions may also interact with each other with rate constant ε_{2PL} per unit time to produce a lethal (i.e., irrepairable) lesion. The latter process will be called binary misrepair.

2) Another fate of a potentially lethal lesion is its fixation, i.e. being made lethal, by such processes as trypsinization and, perhaps, the movement of the cell through "fixation" points in the cell cycle or the addition of a repair-inhibiting drug after irradiation.

3) In order to write an expression for cell survival, we assume, in this version of the model, a Poisson distribution in the number of lesions per cell after the available repair time has elapsed.

4) The mean number of lesions per cell is assumed to vary with time in the same way (i.e., follow the same differential equations) as the lesions in each individual cell.

5) It is assumed that the mean numbers of both kinds of lesion are formed at rates proportional to the absorbed dose rate. Thus, for a given dose rate, $\dot{D}$, the mean numbers of lethal and potentially lethal lesions formed per cell per unit time are $\eta_L\dot{D}$ and $\eta_{PL}\dot{D}$, respectively, where η_L and η_{PL} are the rates of production per unit absorbed dose of the two kinds of lesions.

6) We assume that the rate of repair per lesion is not dependent on the number of lesions present (i.e., there is no saturation in the repair process), and that the probability for potentially lethal lesion interaction depends not on how far apart the lesions were at the time of

creation, but on the square of their overall concentration at any given time.

Symbolically, the model is presented in Figure 2 with the parameters shown as defined above for the lethal and potentially lethal lesions. The primed parameters at the bottom of the figure are included to account for an extension of the model to radiobiological results obtained when the cells are placed in hypertonic solution after radiation (e.g., 7, 10). It is assumed in this case that other lesions normally repaired correctly are involved. This will be discussed in more detail in a later section.

The Differential Equations for Arbitrary Dose Rate

A. During the irradiation.

With the above assumptions, we can write the two differential equations governing the time rate of change of the mean numbers of potentially lethal, $n_{PL}(t)$, and lethal, $n_L(t)$, lesions during the irradiation period[2]:

$$\frac{dn_{PL}(t)}{dt} = \eta_{PL}\,\dot{D} - \varepsilon_{PL}\,n_{PL}(t) - \varepsilon_{2PL}\,n_{PL}^2(t) \tag{1}$$

$$\frac{dn_L(t)}{dt} = \eta_L\,\dot{D} + \varepsilon_{2PL}\,n_{PL}^2(t) \tag{2}$$

The initial conditions are that $n_{PL}(0) = n_L(0) = 0$; i.e. no lesions are assumed to be present at the start of the irradiation. The details for solving these equations are left to Appendix I. The solutions for the time dependence of the mean numbers of potentially lethal and lethal lesions are:

$$n_{PL}(t) = \frac{2\eta_{PL}\dot{D}(1 - e^{-\varepsilon_o t})}{\varepsilon_o + \varepsilon_{PL} + (\varepsilon_o - \varepsilon_{PL})e^{-\varepsilon_o t}} \tag{3}$$

where $\varepsilon_o = (\varepsilon_{PL}^2 + 4\ \varepsilon_{2PL}\ \eta_{PL}\dot{D})^{1/2}$ (4)

and

$$n_L(t) = \eta_L D + \varepsilon \ln \left[\frac{2\varepsilon_o}{\varepsilon_o + \varepsilon_{PL} + (\varepsilon_o - \varepsilon_{PL}) e^{-\varepsilon_o t}} \right] + \frac{(\varepsilon_o - \varepsilon_{PL})^2 t}{4\varepsilon_{2PL}} - n_{PL}(t) \quad (5)$$

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

B. After the irradiation

If we assume the irradiation stops at time T, we have similar equations for the repair and interaction of lesions in the post-irradiation period, but, of course, without the source terms involving the dose rate:

$$\frac{dn_{PL}(t)}{dt} = -\ \varepsilon_{PL}\ n_{PL}(t) - \varepsilon_{2PL}\ n_{PL}^2(t) \quad (6)$$

$$\frac{dn_L(t)}{dt} = \varepsilon_{2PL}\ n_{PL}^2(t) \quad (7)$$

where the initial conditions are

$n_{PL}(T)$ = the value of n_{PL} in equation (3) with t = T

and

$n_L(T)$ = the value of n_L in equation (5) with t = T.

The details for solving these equations are in Appendix II. The solutions are:

$$n_{PL}(t) = N_{PL}\, e^{-\varepsilon_{PL} t_r} \Big/ \left[1 + N_{PL}/\varepsilon(1-e^{-\varepsilon_{PL} t_r})\right] \tag{8}$$

and

$$n_L(t) = N_L + N_{PL}(1 + N_{PL}/\varepsilon)(1-e^{-\varepsilon_{PL} t_r})\ [1 + N_{PL}/\varepsilon(1-e^{-\varepsilon_{PL} t_r})]$$

$$- \varepsilon \ln [1 + N_{PL}/\varepsilon(1-e^{-\varepsilon_{PL} t_r})] \tag{9}$$

with $N_L = n_L(T)$ from equation (5), $N_{PL} = n_{PL}(T)$ from equation (3), t_r = the time available for repair after the end of the exposure, and $\varepsilon = \varepsilon_{PL}/\varepsilon_{2PL}$.

The Survival Equation

To calculate survival at time $t = T + t_r$, the time after which no more repair can occur and the fate of the cell has been determined, we make the assumption that the total mean number of lethal lesions per cell is the sum of the lethal and potentially lethal lesions per cell. That is, we assume that all potentially lethal lesions still present at the end of the available repair time, t_r, are "fixed" (i.e., made lethal).

Then for a given repair time, t_r, the total mean number of lethal lesions per cell is

$$n_{TOT}(T + t_r) = n_L(T + t_r) + n_{PL}(T + t_r) \tag{10}$$

Using the Poissonian assumption for the distribution of lethal lesions per cell, we write the survival as the probability that a cell has no lethal lesion:

$$S = e^{-n_{TOT}(T+t_r)} = e^{-n_L(T+t_r)-n_{PL}(T+t_r)} \tag{11}$$

Substituting equations (9) and (8) for n_L and n_{PL}, respectively, we obtain

$$S = e^{-(N_L+N_{PL}) + \varepsilon \ln[1+N_{PL}/\varepsilon(1-e^{-\varepsilon_{PL}t_r})]}$$

$$= e^{-N_{TOT}} [1 + N_{PL}/\varepsilon(1-e^{-\varepsilon_{PL}t_r})]^{\varepsilon} \tag{12}$$

where N_L = number of lethal lesions at the end of the exposure time

N_{PL} = number of potentially lethal lesions at the end of the exposure time

ε = $\varepsilon_{PL}/\varepsilon_{2PL}$

t_r = repair time available after the end of exposure

N_{TOT} = $N_L + N_{PL}$

The values of N_{PL} and N_L are obtained from equations (3) and (5), respectively, with t = T, the exposure time.

The survival can then be written in general terms of the dose rate, D, the absorbed dose, D = DT, and the mean available repair time, t_r, as

$$S(D,\dot{D},t_r) = e^{-N_{TOT}(D/\dot{D})}\left[1 + \frac{N_{PL}(D/\dot{D})}{\varepsilon}\left(1 - e^{-\varepsilon_{PL}t_r}\right)\right]^{\varepsilon} \qquad (13)$$

where $N_{TOT}(D/\dot{D}) = n_{PL}(T) + n_L(T)$ from equations (3) and (5),

$N_{PL}(D/\dot{D}) = n_{PL}(T)$ from equation (3)

t_r = the mean time available for repair after the end of the exposure.

For long repair time, the exponential involving t_r becomes very small. For cellular studies utilizing stationary (plateau) phase cells left in conditioned medium for very long periods after irradiation (delayed plating experiments), the available repair time can be considered to be long enough ($t_r >> 1/\varepsilon_{PL}$) so that this term can be neglected. Then, the survival equation becomes

$$S(D,\dot{D},\infty) = e^{-N_{TOT}(D/\dot{D})}\left[1 + \frac{N_{PL}(D/\dot{D})}{\varepsilon}\right]^{\varepsilon} \qquad \text{(delayed plating)} \qquad (14)$$

<u>Comparison with experiment - determination of values for parameters</u>

Experiments at a variety of dose rates using plateau phase C3H10T1/2 cells have been reported by Wells and Bedford (11). Enough repair time was allowed after the end of the exposure so that equation (14) applies. An analysis was made to determine values of the four parameters in the model: n_L, n_{PL}, ε_{PL}, ε_{2PL} for this cell line. The parameter n_L is simply the reciprocal of the D_0 for the exponential curve obtained at very low dose rates (see next section) ($n_L = 1/7.32 = 0.1366\ Gy^{-1}$). The values of n_{PL} and $\varepsilon = \varepsilon_{PL}/\varepsilon_{2PL}$ were

obtained by finding a good fit to the high dose rate survival curve using equation (14). The results were $\eta_{PL} = 0.6\ Gy^{-1}$ and $\varepsilon = 9.0$. The value of ε_{PL} was chosen to reflect a characteristic mean repair time of two hours ($\varepsilon_{PL} = 0.5\ hr^{-1}$).

A family of curves using these parameters is shown in Figure 3 for graded dose rates from 5×10^{-3} to 10^4 Gy/hr. Intermediate values, for which the shape of the survival curve is a function of dose rate, are indicated in the figure. At the high and low end of the dose rate range, there is no dependence of the curve shape on dose rate.

The variation of ε_0 on dose rate is shown in Figure 4. For the values of the parameters chosen, the high dose rate region is reached when ε_0 approaches about 4-5 (i.e., around 1 Gy/min).

In Figure 5 we show the survival curves with the above values for the parameters compared with the experimental data of Wells and Bedford (11).

Low dose rate approximation

We will define the low dose rate approximation to hold at those low dose rates such that the survival curves are not a function of dose rate. The condition is

$$\dot{D} \ll \varepsilon_{PL}/\eta_{PL} \qquad \text{low dose rate}$$

A proof with the restriction $\varepsilon \geq 2$ is given in Appendix III. The survival curve becomes simply

$$S = e^{-\eta_L D} \tag{15}$$

High dose rate approximation

We define the high dose rate approximation to hold at those high dose rates such that, again, the survival curves are not a function of dose rate. The condition is

$$\dot{D} \gg \varepsilon_{PL}^2/(4\eta_{PL}\varepsilon_{2PL}) \qquad \text{high dose rate}$$

A proof of this with a restriction on the exposure time $T \ll 2/\varepsilon_0$ is given in Appendix IV. If these conditions are met, the survival equation reduces to

$$S = e^{-(\eta_L + \eta_{PL})D}\left[1 + \frac{\eta_{PL}D}{\varepsilon}\left(1 - e^{-\varepsilon_{PL}t_r}\right)\right]^{\varepsilon} \tag{16}$$

We note this is just equation (13) with $N_{TOT} = (\eta_L + \eta_{PL})D$ and $N_{PL} = \eta_{PL}D$. This is the solution obtained previously (12) with the assumption that the exposure time was short compared to the repair time; that is, all of the lesions created by the radiation were present at the end of the exposure.

Linear-Quadratic Approximation at Low Dose

An interesting approximation can be made to equation (16) in the region of low doses. If the survival expression is rewritten:

$$-\ln S = (\eta_L + \eta_{PL})\, D - \varepsilon \ln\left[1 + \eta_{PL}\, D/\varepsilon\left(1 - e^{-\varepsilon_{PL}t_r}\right)\right] \tag{17}$$

and the logarithmic term is expanded in a power series valid for small values

of D and if we keep only the first two terms of the expansion (those involving D and D^2), we obtain:

$$-\ln S = \eta_L D + \eta_{PL} D - \varepsilon \left[\eta_{PL} D/\varepsilon (1-e^{-\varepsilon_{PL} t_r}) - \frac{\eta_{PL}^2 D^2}{2\varepsilon^2} (1-e^{-\varepsilon_{PL} t_r})^2 \right] \tag{18}$$

The restriction on D is $D \ll 3\varepsilon/[2\eta_{PL}(1-e^{-\varepsilon_{PL} t_r})]$.

Simplifying, we obtain

$$-\ln S = (\eta_L + \eta_{PL} e^{-\varepsilon_{PL} t_r}) D + \frac{\eta_{PL}^2}{2\varepsilon} (1 - e^{-\varepsilon_{PL} t_r})^2 D^2 \tag{19}$$

We see that the expression is linear-quadratic in the absorbed dose. We identify the linear and quadratic coefficients, α and β, respectively:

$$\alpha = \eta_L + \eta_{PL} e^{-\varepsilon_{PL} t_r} \tag{20}$$

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

Thus, we see that linear-quadratic dependence on the dose is a special case of the LPL model and is valid only at those low doses where inclusion of only the first two terms of the power expansion is justified.

Derivation of the "dose protraction factor" of the linear-quadratic models at low doses

Another interesting low dose approximation can be made. For sufficiently low doses, it is possible to neglect the η_{PL}^2 term in equations (1) and (6). If

this is done, the differential equations (1 and 2) for $0 \leq t \leq T$ become

$$\frac{d\ {}_1n_{PL}(t)}{dt} = \eta_{PL}\dot{D} - \varepsilon_{PL}\ {}_1n_{PL}(t) \tag{22}$$

$$\frac{dn_L(t)}{dt} = \eta_L\dot{D} - \varepsilon_{2PL}\ {}_1n^2_{PL}(t) \tag{23}$$

where ${}_1n_{pL}(t)$ is the new function satisfying the modified differential equation.

The first can be solved to yield

$${}_1n_{PL}(t) = \eta_{PL}\dot{D}/\varepsilon_{PL}(1-e^{-\varepsilon_{PL}t}) \tag{24}$$

and so at the end of the exposure (t = T),

$${}_1n_{PL}(T) = N_{PL} = \eta_{PL}\dot{D}/\varepsilon_{PL}(1-e^{-\varepsilon_{PL}T}) \tag{25}$$

For $t \geq T$, we neglect the quadratic term in equation (6) and the rate of change of the potentially lethal lesions, ${}_2n_{PL}(t)$, becomes

$$\frac{d\ {}_2n_{PL}(t)}{dt} = -\varepsilon_{PL}\ {}_2n_{PL}(t) \tag{26}$$

which is immediately solved to be

$$ {}_2n_{PL}(t) = N_{PL}\, e^{-\varepsilon_{PL} t} \tag{27} $$

The differential equation for the lethal lesions in this time interval remains

$$ \frac{dn_L(t)}{dt} = \varepsilon_{2PL}\, {}_2n_{PL}^2(t) \tag{28} $$

The number of lethal lesions at the end of the available repair time, t_r, can be obtained by integrating over the total time:

$$ n_L(t_r) = \eta_L \dot{D} \int_0^T dt' + \varepsilon_{2PL} \left[\int_0^T {}_1n_{PL}^2(t')dt' + \int_T^{T+t_r} {}_2n_{PL}^2(t')dt' \right] \tag{29} $$

where ${}_1n_{PL}(t')$ and ${}_2n_{PL}(t')$ are given by equations (25) and (27), respectively.

This is immediately integrated to give

$$ n_L(t_r) = \eta_L D + \frac{n_{PL}^2 D^2}{2\varepsilon} \cdot \frac{2}{(\varepsilon_{PL} T)^2} \left[\varepsilon_{PL} T + e^{-\varepsilon_{PL} T} - 1 - 1/2 \left(e^{-\varepsilon_{PL} t_r} - e^{-\varepsilon_{PL}(T+t_r)} \right)^2 \right] \tag{30} $$

Now, in addition, if we assume very long repair times are available, i.e., t_r is very large compared to $1/\varepsilon_{PL}$, the last term in the brackets can be neglected and we have for the total mean number of lesions, n_{TOT}:

$$\lim_{t_r \to \infty} \left[2n_{PL}(t_r) + n_L(t_r) \right] = \eta_L D + \frac{\eta_{PL}^2 \; D^2}{2\varepsilon} \cdot \frac{2}{(\varepsilon_{PL}T)^2} \left(\varepsilon_{PL}T + e^{-\varepsilon_{PL}T} - 1 \right)$$

$$= \eta_L D + \eta_{PL}^2 \; D^2/_{2\varepsilon} \; G(T) \qquad (31)$$

where $$G(T) = \frac{2}{(\varepsilon_{PL}T)^2} \left(\varepsilon_{PL}T + e^{-\varepsilon_{PL}T} - 1 \right)$$

We see that we obtain the "dose protraction" or "dose rate" factor G(T) which occurs in the time dependent theory of Dual Radiation Action (2), the Accumulation Model (13), and the Molecular Theory (9,14). This function also appears in an earlier theoretical treatment by Lea and Catcheside (15) describing chromosome breaks and exchanges. As pointed out by Lea and Catcheside (15) and by Lea (16), it appears when the equation $dn/dt = K\dot{D} - k_1 n$ (for the breaks) during exposure and $\int k_2 n^2 dt$ (for the exchanges) during and after exposure are solved with subsequent allowance for long rejoining times to elapse.

Thus, the present formulation includes within it the previous dose rate formulations yielding linear-quadratic dependence on the dose and the "dose protraction factor" as special cases which are valid at low doses only.

Behavior of the survival expresssion at high doses

We can differentiate equation (17) with respect to absorbed dose D and take the limit as D approaches infinity, obtaining

$$\lim_{D\to\infty}\left[\frac{d(-\ln S)}{dD}\right] = \lim_{D\to\infty}\left[\eta_L + \eta_{PL} - \frac{\eta_{PL}(1-e^{-\varepsilon_{PL}t_r})}{1+\eta_{PL}D/\varepsilon(1-e^{-\varepsilon_{PL}t_r})}\right]$$
$$= \eta_L + \eta_{PL} \tag{32}$$

We note that the slope of the survival curve approaches a limit given by $\eta_L + \eta_{PL}$, the rate per unit dose of the production of the sum of the lethal and potentially lethal lesions.

Sublethal and Potentially Lethal Damage

Operational definitions of sublethal and potentially lethal damage can be stated as follows: Sublethal damage (SLD) is that damage that is repaired between doses in a split dose experiment or during exposure in a low dose rate experiment. Potentially lethal damage (PLD) is that damage that is repaired after the end of the radiation exposure (or exposures). Applying these definitions in the consideration of the above formulation, we see that both types of damage are accounted for and, in addition, they both arise from the same type of repairable lesion, designated here potentially lethal lesions. Thus, in the LPL model, there is no fundamental difference between SLD and PLD repair, the former occurring during or between irradiations and the latter occurring after the final irradiation. All repairable damage is characterized by one class of repairable lesions with mean repair time $1/\varepsilon_{PL}$. Both kinds of damage are accumulated in the sense that it takes two lesions to interact to form a lethal lesion. In addition, however, these lesions can be "fixed" by cellular processes probably at various points throughout the cell cycle. Such fixation may be the reason that many cell lines show little or no repair of

potentially lethal damage in exponential growth phase. For cells in cycle, in this view, the damage is fixed at "fixation points" in the cycle before having the opportunity to be repaired. Roughly the same amount of damage is repaired in the growth medium independently of when the trypsinization occurs. For cell lines in exponential growth that show repair of PLD, the trypsinization procedure itself may be a mild inhibitor of damage repair, thus "fixing" some lesions which, if left undisturbed a longer time, might have been repaired correctly.

Interpretation of "Conditioned" and "Fresh" Medium Experiments

These ideas can be used to interpret experimental results on the repair of PLD in Ehrlich ascites tumor cells in plateau phase obtained by Iliakis (17). Using parametric values of $\eta_L = 0.2\ Gy^{-1}$, $\eta_{PL} = 1.1\ Gy^{-1}$, $\varepsilon = 10$ and $\varepsilon_{PL} = 0.5\ hr^{-1}$, we first calculate from equations (8) and (9), the time dependence of the numbers of the two kinds of lesions, η_{PL} and η_L, respectively. This is shown in Figure 6 for an exposure of 7 Gy of x-irradiation. Here and in succeeding considerations, we are assuming the high dose rate approximation applies so that $N_L = \eta_L D$ and $N_{PL} = \eta_{PL} D$. The calculations were made for two experimental conditions: for the cells in conditioned or "C"-medium and fresh or "F"-medium. The conditioned medium is the same medium in which these plateau phase cells were being maintained in suspension immediately before the irradiation. The fresh medium is growth medium which causes the cells to enter the cell cycle and start proliferating. The calculations were made with the assumption that, for the F-medium case, a fixation point of some kind occurred at 3 hours postirradiation, and all remaining potentially lethal lesions became lethal and no more repair could occur. A comparison of the calculated survival time dependence with the experimental results of Iliakis for the two

experimental conditions is shown in Figure 7. The time in the experiments was the time interval after the completion of the irradiation exposure before the repair inhibiting drug β-araA (β-arabinofuranosyladenine) was added to the cultures. We assume complete fixation of remaining potentially lethal lesions by the drug at the time of its application. The experimental data can be interpreted as indicating that in fresh medium there is an "effective" repair time of 3 hours after which the lesions are fixed and no more repair is possible.

Split-Dose: Repair of Sublethal Damage

We assume the same mathematical formulation applies to the interpretation of split-dose experiments. For irradiations D_1 and D_2 separated by a time interval Δt, new lesions produced by the second dose add to the remaining lesions not yet repaired from the first dose and produce a new total number of lesions per cell. It is convenient to write the number of lesions as a function of the number of initial lesions N formed by a dose, D, and the repair time interval allowed, t. Thus $n_L(t) = n_L(N_L(D),t)$ and $n_{PL}(t) = n_{PL}(N_{PL}(D),t)$.

After a time interval Δt, a first dose D_1 will yield

$$n_L(\Delta t) = n_L(N_L(D_1), \Delta t) \text{ and } n_{PL}(\Delta t) = n_{PL}(N_{PL}(D_1),\Delta t) \tag{33}$$

Immediately after the second irradiation, D_2, we have

$$n_L(D_1,D_2,\Delta t) = n_L(n_L(N_L(D_1),\Delta t)+N_L(D_2),0) \tag{34}$$

and

$$n_{PL}(D_1,D_2,\Delta t) = n_{PL}(n_{PL}(N_{PL}(D_1),\Delta t)+N_{PL}(D_2),0) \tag{35}$$

After a repair time t_r allowed after the second dose, we have

$$n_L(D_1,D_2,\Delta t,t_r) = n_L(n_L(N_L(D_1),\Delta t)+N_L(D_2),t_r) \tag{36}$$

and

$$n_{PL}(D_1,D_2,\Delta t,t_r) = n_{PL}(n_{PL}(N_{PL}(D_1),\Delta t)+N_{PL}(D_2),t_r) \tag{37}$$

Here, repair is occurring both within the fraction interval Δt and after the second exposure, during a time t_r.

Using the same parameters as in the above example, the calculated time course of lesions is shown in Figure 8 for conditioned medium (top panel) and fresh medium (bottom panel), in a split-course of 2 Gy + 5 hours + 2 Gy. After three hours in fresh medium, the time interval does not affect the final survival (which is $S = e^{-n_L-n_{PL}}$). Thus, split dose experiments of stationary phase cells in fresh medium yield a measure of the repair available to the "time to fixation" in the fresh medium rather than the true repair kinetics of the cellular lesions.

Inclusion of the Effects of Hypertonicity

In experiments with cells placed in hypertonic solution after irradiation, it has been found that (1) the slope of the survival curve is steeper than if β-araA is used as repair inhibitor (7, 18), and (2) the repair kinetics is much faster than found after experiments using β-araA (7, 18) or after split-dose experiments (10). The interpretation of these results within the framework of the LPL model requires the assumption that a new class of lesions with faster repair kinetics ($t_{1/2} \approx$ 3-10 minutes) is involved. Figure 2 (bottom) shows these lesions as a distinctly separate class with primed parameters. The assumptions are made that (1) there is no interaction between these lesions (i.e., first order repair kinetics applies) and (2) the hypertonic treatment "fixes" the lesions that are left unrepaired . In this case, for the high dose rate situation, the value of n_{TOT} in equation (10) includes a third term, n_{PL}':

$$n_{PL}' = \eta_{PL}' e^{-\varepsilon_{PL}' t_r} D \tag{38}$$

The primed coefficients are η_{PL}' = the production rate per unit dose for the new lesions

ε_{PL}' = the repair rate per unit time for the new lesions

Variation of Radiation Quality in "Track Segment" Experiments

In "track segment" experiments, a uniform fluence of charged particles, ϕ, with a constant single-valued LET, L, is assumed to traverse the cell population. In this case, $D = kL\phi$, where k is a proportionality constant whose value depends on the units of D, ϕ and L. As is customarily done, we now define a probability for lesion production per unit fluence called a cross section, σ, which has the dimension of an area. Then for the lethal and

potentially lethal lesions, respectively, we have

$$\eta_L = \eta_L D = k\eta_L L\phi = \sigma_L \phi \tag{39}$$

$$\eta_{PL} = \eta_{PL} D = k\eta_{PL} L\phi = \sigma_{PL} \phi \tag{40}$$

From these equations, the relationships between the η's and σ's are:

$$\eta_L = \sigma_L/(kL) \tag{41}$$

$$\eta_{PL} = \sigma_{PL}/(kL) \tag{42}$$

Eq. (16) then becomes

$$S = e^{-(\sigma_L + \sigma_{PL})D/(kL)} \left[1 + \sigma_{PL} \quad D(1 - e^{-\varepsilon_{PL} t_r})/(kL\varepsilon)\right]^{\varepsilon} \tag{43}$$

With the absorbed dose, D, expressed in Gy, the LET, L, expressed in keV/μm of water and the cross section for lesion production expressed in μm^2, $k = 0.16$ Gyμm^3/keV.

We note immediately that there is a reciprocal dependence on the LET in two of the terms in equation (43); the cross sections, however, also depend on the radiation quality of the particle beam.

Assumptions for the Cross Sections

We make the following assumptions in order to calculate expressions for the cross sections:

1. Biochemical prelesions are formed by clusters of ionizations Poissionly distributed along the tracks of charged particles traversing the cell nucleus. The mean distance between prelesions is assumed to be λ; thus, the mean number of prelesions per unit length is $1/\lambda$.
2. The distance of these prelesions from the track trajectory is small compared to their separation.
3. There are on the average n "critical" regions (targets) of average length X_0 randomly distributed along each track through the cell nucleus.
4. An immediately lethal (i.e., irrepairable) lesion is caused when two or more prelesions occur within a critical region of average extension X_0 along the track.
5. A potentially lethal lesion can arise from an isolated prelesion.
6. The cell nuclei have an average radiobiologically effective cross section σ_0 presented to the particle beam.

Now using the Poissonian assumption for the distribution of prelesions, we can write the probabilities for prelesion formation in terms of the mean number of prelesions per critical region, X_0/λ:

$P_o = e^{-X_0/\lambda}$ = probability of finding no prelesions in a distance X_0 along the track

$P_1 = (X_0/\lambda)e^{-X_0/\lambda}$ probability of finding one and only one prelesion in a distance X_0 along the track

$P_{\geq 2} = 1 - P_0 - P_1$ = probability of finding two or more prelesions in a distance X_0 along the track

$$= 1 - e^{-X_0/\lambda} (1 + X_0/\lambda)$$

Now $1 - P_{\geq 2}$ = probability of failing to find two or more prelesions in X_0

and $(1 - P_{\geq 2})^n$ = probability of failing to find two or more prelesions in <u>any</u> n randomly selected distances X_o along the track. The probability, then of finding <u>at least one lethal lesion</u> (i.e., two or more prelesions within at least one critical site) along a track is

$$P_L = 1 - (1 - P_{\geq 2})^n \tag{44}$$

The cross section, σ_L, has originally been defined as the probability of lethal lesion production per unit fluence, but since by definition only one lethal lesion is necessary to kill the cell, σ_L should be reinterpreted as the probability per unit fluence of <u>one or more</u> lethal lesions being produced. At high LET, there is a greater probability of more than one lethal lesion to be produced by each track. The total number of lethal lesions does not saturate but the probability of <u>one or more</u> lethal lesion being formed does saturate. This is reflected by the above equation.

We write the cross section, σ_L, for cell killing via the direct lethal lesion process as follows:

$$\sigma_L = \sigma_0[1-(1-P_{\geq 2})^n] = \sigma_0\{1 - [e^{-X_0/\lambda}(1 + X_0/\lambda)]^n\} \tag{45}$$

The mean number of isolated prelesions within critical sites per track is P_1 and for an average of n critical sites per traversed nucleus, the number of isolated prelesions within critical sites per track length through the nucleus is nP_1. The cross section, σ_{PL}, for potentially lethal lesion formation is assumed to be proportional to nP_1:

$$\sigma_{PL} = F_{PL}\ \sigma_o\ nP_1 = F_{PL}\ \sigma_o\ n\ X_0/\lambda\ e^{-X_0/\lambda} \tag{46}$$

Here F_{PL} is the probability that, given a prelesion within a critical site, it will remain to become a potentially lethally lesion. This factor will depend on the chemical environment within the cell nucleus (e.g., concentration of oxygen and sulfhydrals). Thus, this is where fast chemical restitution processes and the competition between oxygen fixation and hydrogen donation plays a role.

At low LET, i.e., for large λ, there are few prelesions per unit track length. To see the dependence of the cross sections at low LET, we expand equations (45) and (46), keeping only the terms of lowest order in X_0/λ and obtain

$$\sigma_L \simeq \sigma_o\ n\ X_0^2/(2\lambda^2) \qquad \text{(low LET approximation)} \tag{47}$$

$$\sigma_{PL} \simeq F_{PL}\ \sigma_o\ n\ X_0/\lambda \qquad \text{(low LET approximation)} \tag{48}$$

Thus, at low LET, σ_L increases as the square of $1/\lambda$ and σ_{PL} increases linearly with $1/\lambda$. To the extent that $1/\lambda$ is directly proportional to the LET

of the particles, these statements can also be made about the variations of the cross sections with LET.

The reason for the quadratic dependence of σ_L at low LET is traceable directly to the assumption that the irrepairable lesions are caused by (at least) two prelesions in a critical site. This is similar to the assumption of the "site model" in the Theory of Dual Radiation Action (2). The size of the critical site X_0, however, appears only in the ratio X_0/λ, and so knowledge of its magnitude must await the determination of λ, the mean distance between prelesions formed along the track. If, for some intermediate value of LET, $\lambda \simeq 0.1\ \mu m$, X_o will turn out to be about 10 nanometers. One suggestion is that X_0 is the mean distance traversed by a charged particle through a strand of DNA.

Lacking physical data on geometrical distribution of ionization clusters and/or relevant chemical lesions around and along particle tracks within a cell nucleus, we make one further assumption, valid only in a limited range of particle effective charge, z^*, and velocity, βc, that X_0/λ is proportional to z^{*2}/β^2:

$$X_0/\lambda = k_0 z^{*2}/\beta^2 \tag{49}$$

Dependence of the cross section on this parameter instead of LET has come from the realization, apparent for some time (19-21) that particles with different z's and the same LET cause different amounts of cell killing. This has lead to the suggestion that z^{*2}/β^2 might be a better parameter than LET to characterize such radiobiological quantities as the OER (oxygen enhancement ratio) of mammalian cells (20). The same idea has been incorporated into the ion-gamma kill model of Katz (5).

The cross sections then become:

$$\sigma_L(z^*,\beta) = \sigma_o\{1-[e^{-k_o z^{*2}/\beta^2})(1 + k_o z^{*2}/\beta^2)]^n\} \tag{50}$$

$$\sigma_{PL}(z^*,\beta) = F_{PL}\ \sigma_o\ nk_o z^{*2}/\beta^2\ e^{-k_o z^{*2}/\beta^2} \tag{51}$$

Fig. 9 shows σ_L and σ_{PL} plotted as a function of z^{*2}/β^2 with the following values for the parameters:

σ_o = 45 μm^2, n = 12

F_{PL} = 0.1 (oxygenated cells), F_{PL} = 0.08 (hypoxic cells)

k_o = 1/4000 (oxygenated cells), k_o = 1/5760 (hypoxic cells)

A comparison is made with best fit values of σ_L and σ_{PL} obtained from survival of T - 1 human kidney cells irradiated with alpha particles (21).

Concluding Remarks

A unified repair model of radiation action has been presented embracing several of the major concepts in theoretical radiobiology which have been developed over the last few decades. The "interaction-of-lesions" idea appeared in the early work on chromosome aberrations and was incorporated into the model of chromosome misjoining of Lea and Catcheside (15) as well as the models of cell lethality of Neary (4), Kellerer and Rossi (2,3), Roesch (13), Chadwick and Leenhouts (9,14) and Tobias (1). The ideas of (binary) misrepair, (linear) fixation, irrepairable lesions caused by single tracks, at least two different kinds of repairable lesions, the separability in time of single track and multi-track events, the high LET effect being due to higher statistical

probability of two or more "lesions" occurring in a critical site at higher ionization density are all combined to produce a model that appears to be compatable with a considerable amount of experimental data.

One major idea not a part of the model in its present form is that of repair-saturation. As has been shown in the development of several models (23-25) and as recently reviewed by Goodhead (26), repair saturation can also explain very effectively the shoulder on survival curves. Evidence exists at high doses that repair of double strand breaks shows the characteristics of saturation (27). It has yet to be established, however, what role, if any, saturation phenomena play in leading to cell lethality at doses less than 7 Gy where the shoulders of survival curves appear.

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FOOTNOTES

1. These characteristics are not unique to this model. They also apply to the RMR model (1) and the mathematical form of the "modified single hit-multitarget" model with the target number equal to two.

2. The quadratic terms in these equations can be considered to arise from the assumption that the probability per unit time for interaction of the potentially lethal lesions within any cell is proportional to the number of possible pairwise interactions of lesions,

$$\binom{n_i}{2} = 1/2 n_i (n_i - 1)$$

where n_i is the number of such lesions in the ith cell. We note that such pairwise interaction of lesions is also a fundamental assumption of the Theory of Dual Radiation Action of Kellerer and Rossi (2) (in that formulation, they are called sublesions), the accumulation model of Roesch (13) and the Molecular Theory of Chadwick and Leenhouts (9). We assume further that the lesions follow a Poissonian distribution among the cells, with mean number (or expectation value) $\bar{n}$ per cell. The value of $\bar{n}$ varies as a function of time. The quadratic terms in equations (1) and (2) are obtained by assuming that the rate of potentially lethal lesion interaction is proportional to $\overline{n(n-1)}$ which we note is equal to $\overline{n^2} - \bar{n}$. It is easy to show that $\overline{n^2} = \bar{n}(\bar{n} + 1)$ for a Poisson distribution, so that $\overline{n^2} - \bar{n} = \bar{n}^2$. Thus, we have written the third term on the right in equation (1) and the second term on the right in equation (2) in terms of the square of the mean value of the potentially lethal lesions per cell, $\bar{n}^2$, instead of $\overline{n(n-1)}$. The author is indebted to Professor D. Harder for a conversation regarding this point.

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FIGURE CAPTIONS

Figure 1. Evolution of important events leading to cell lethality in the LPL model. Ionization events in the physical domain lead to radicals in the chemical domain which in turn lead to prelesions in DNA still in the chemical domain. If these are in close proximity, they lead to lethal (irrepairable) lesions. If they are isolated, they can, if not restituted, lead to potentially lethal (repairable) lesions. The latter in the biological domain can either interact to form a lethal lesion (binary misrepair), can be "fixed" at some point in the cell cycle, or can be correctly repaired.

Figure 2. Diagrammatic representation of the LPL model and designation of parameters in the biological time frame. The η_L and η_{PL} are the rates per unit absorbed dose for production of the lethal and potentially lethal lesions, respectively. The ε_{PL} and ε_{2PL} are the rates per unit time of correct repair and binary misrepair, respectively, for the potentially lethal lesions. The primed parameters refer to an entirely new class of lesions which can be "fixed" by hypertonic treatment.

Figure 3. A family of survival curves as calculated from the LPL model. Values of the parameters used are given in the text. At high and low dose rates, the survival curves become independent of dose rate. Curves a through h denote values in the midrange of dose rates where the survival curves are a function of dose rate.

Figure 4. Dependence of ε_0 on the dose rate. Low dose rate conditions occur when ε_0 is constant and equal to ε_{PL}. High dose rate conditions occur when ε_0 reaches a value of about 5 for the values of the model parameters chosen.

Figure 5. Comparison of survival curves calculated from the LPL model (solid curves) and the experimental data from C3H 10T1/2 density-inhibited cells irradiated with ^{137}Cs gamma rays obtained by Wells and Bedford (11). Parameters in the model are given in the text.

Figure 6. A comparison of the time course of the mean numbers of lethal (n_L) and potentially lethal (n_{PL}) lesions in C, "conditioned," (dashed line) and F, fresh or growth medium (solid line) after an absorbed dose of 7 Gy. A fixation point is assumed after 3 hours in fresh medium; i. e., all remaining potentially lethal lesions are fixed and become lethal at that point.

Figure 7. Cell survival as a function of time in "conditioned" (dashed curve) or fresh (solid curve) medium after an absorbed dose of 7 Gy. Comparison is made with experimental data from Ehrlich ascites tumor cells (17).

Figure 8. Time course of the mean number of lethal (n_L) and potentially lethal (n_{PL}) lesions for a split dose experiment in "conditioned" medium (top) and in fresh medium (bottom). The experimental protocol is assumed to be a dose of 2 Gy followed by a repair period of 5 hours followed by a dose of 2 Gy.

Figure 9. Lesion production cross sections, σ_L and σ_{PL}, as a function of z^{*2}/β^2 for oxygenated (solid line) or hypoxic (dashed line) cells. Data points were obtained from best fits to cell survival curves obtained with human kidney T-1 cells irradiated with alpha particles and deuterons of various velocities (22). Values of the parameters used to calculate the curves are given in the text.

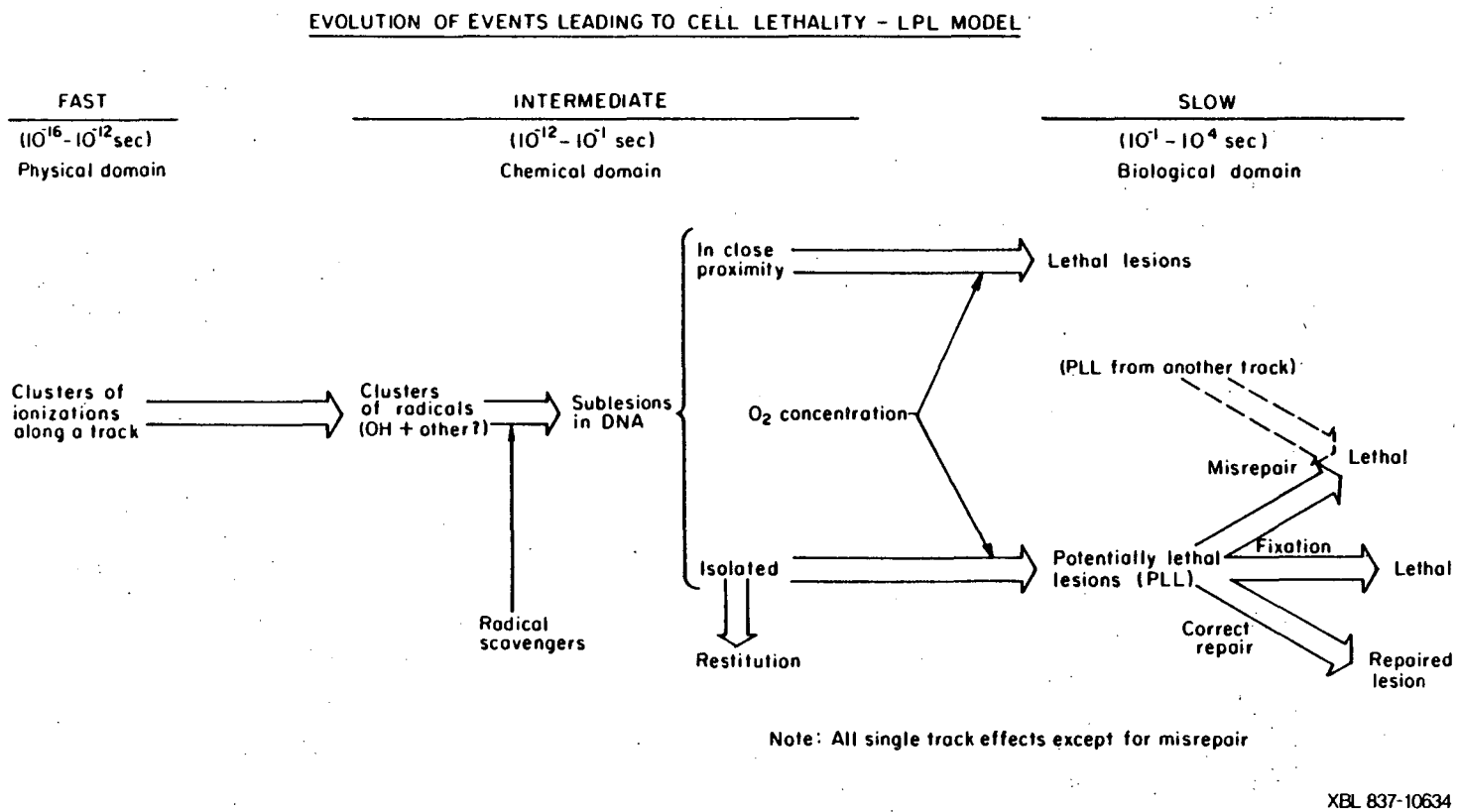
EVOLUTION OF EVENTS LEADING TO CELL LETHALITY - LPL MODEL
FAST
(10^{-16} - 10^{-12} sec)
Physical domain
INTERMEDIATE
(10^{-12} - 10^{-1} sec)
Chemical domain
SLOW
(10^{-1} - 10^{4} sec)
Biological domain
Clusters of ionizations along a track
Clusters of radicals (OH + other?)
Radical scavengers
Sublesions in DNA
In close proximity
Isolated
Restitution
O_2 concentration
Lethal lesions
(PLL from another track)
Misrepair
Lethal
Potentially lethal lesions (PLL)
Fixation
Lethal
Correct repair
Repaired lesion
Note: All single track effects except for misrepair
XBL 837-10634

Figure 1.

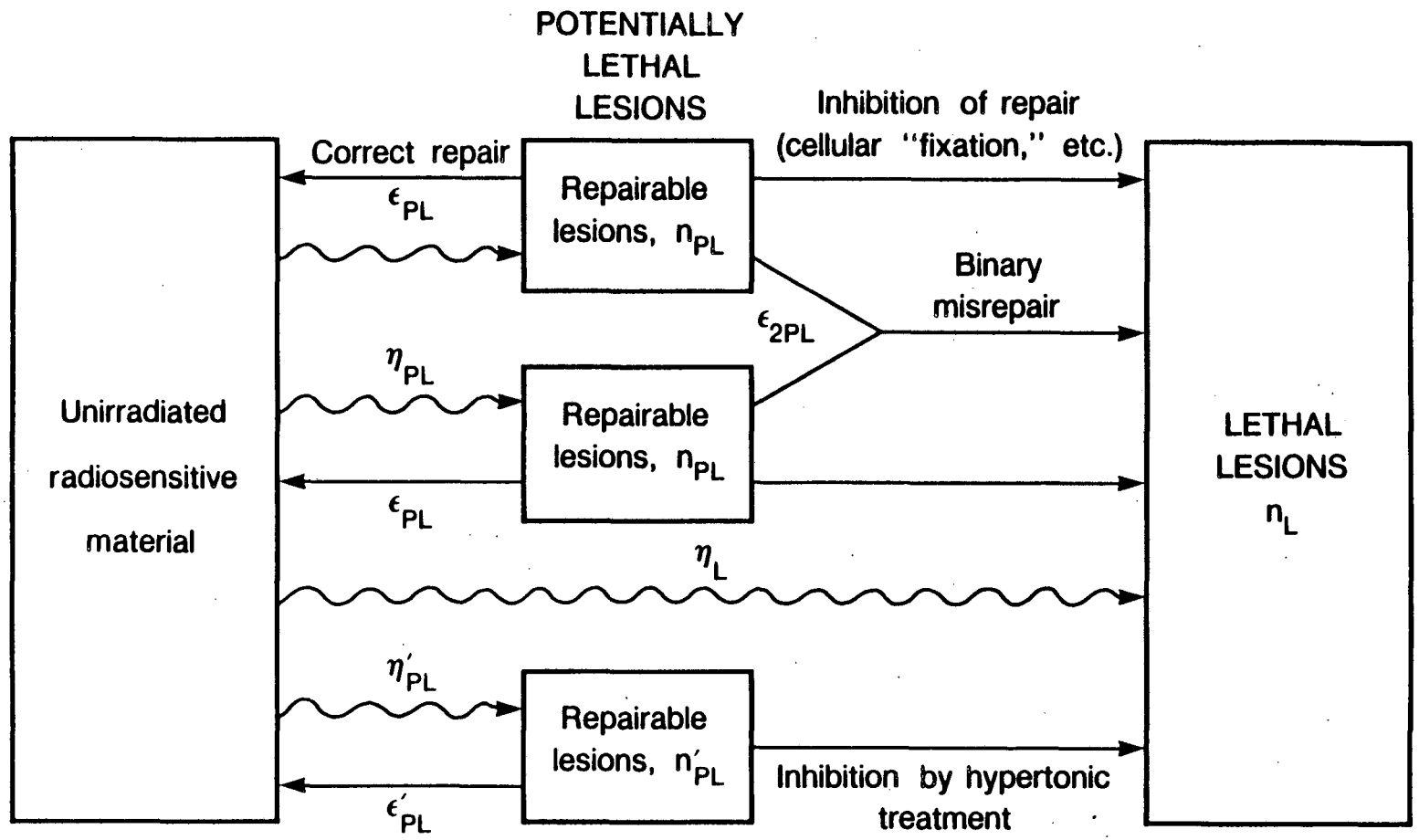

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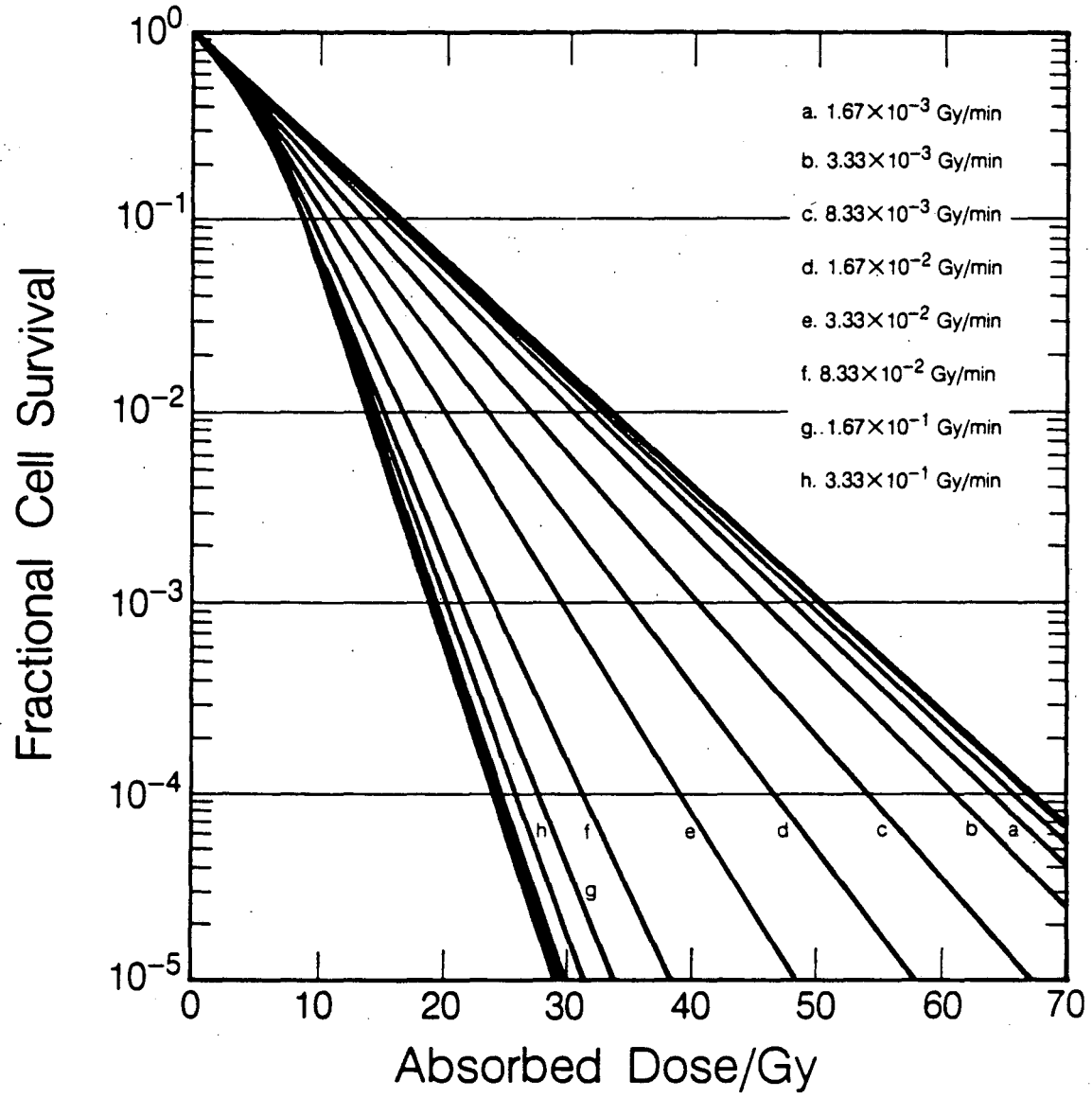
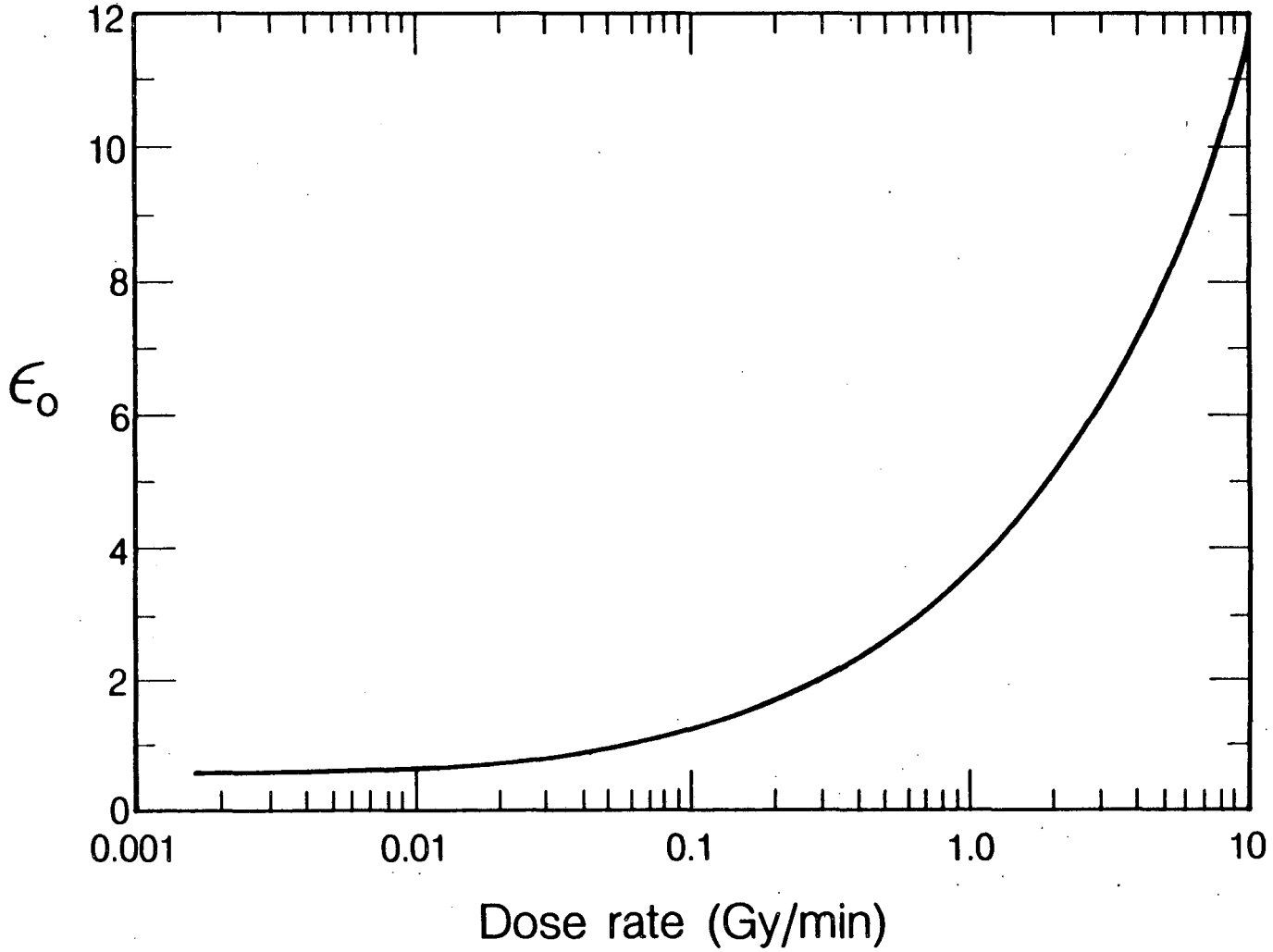
Figure 2.



XBL 844-7694

Figure 3.



XBL 844-7693

Figure 4.

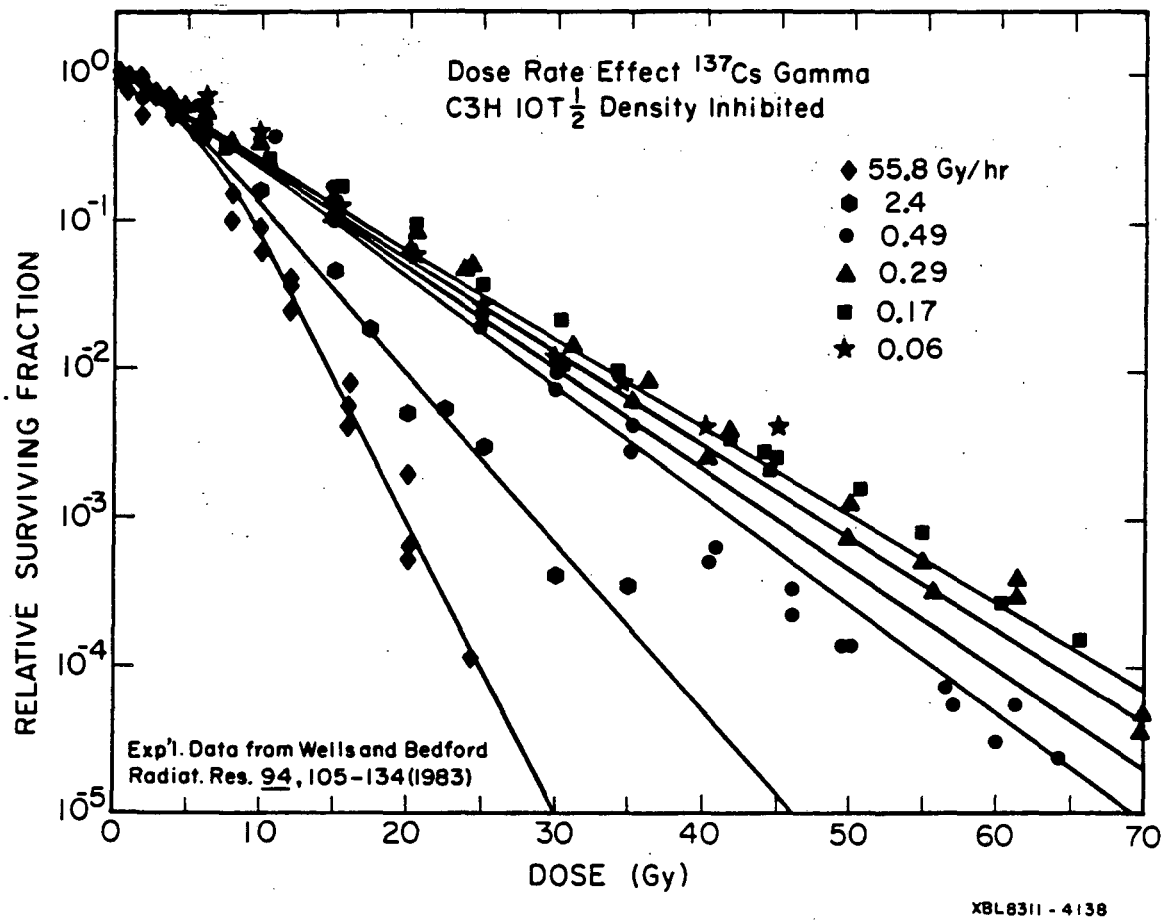
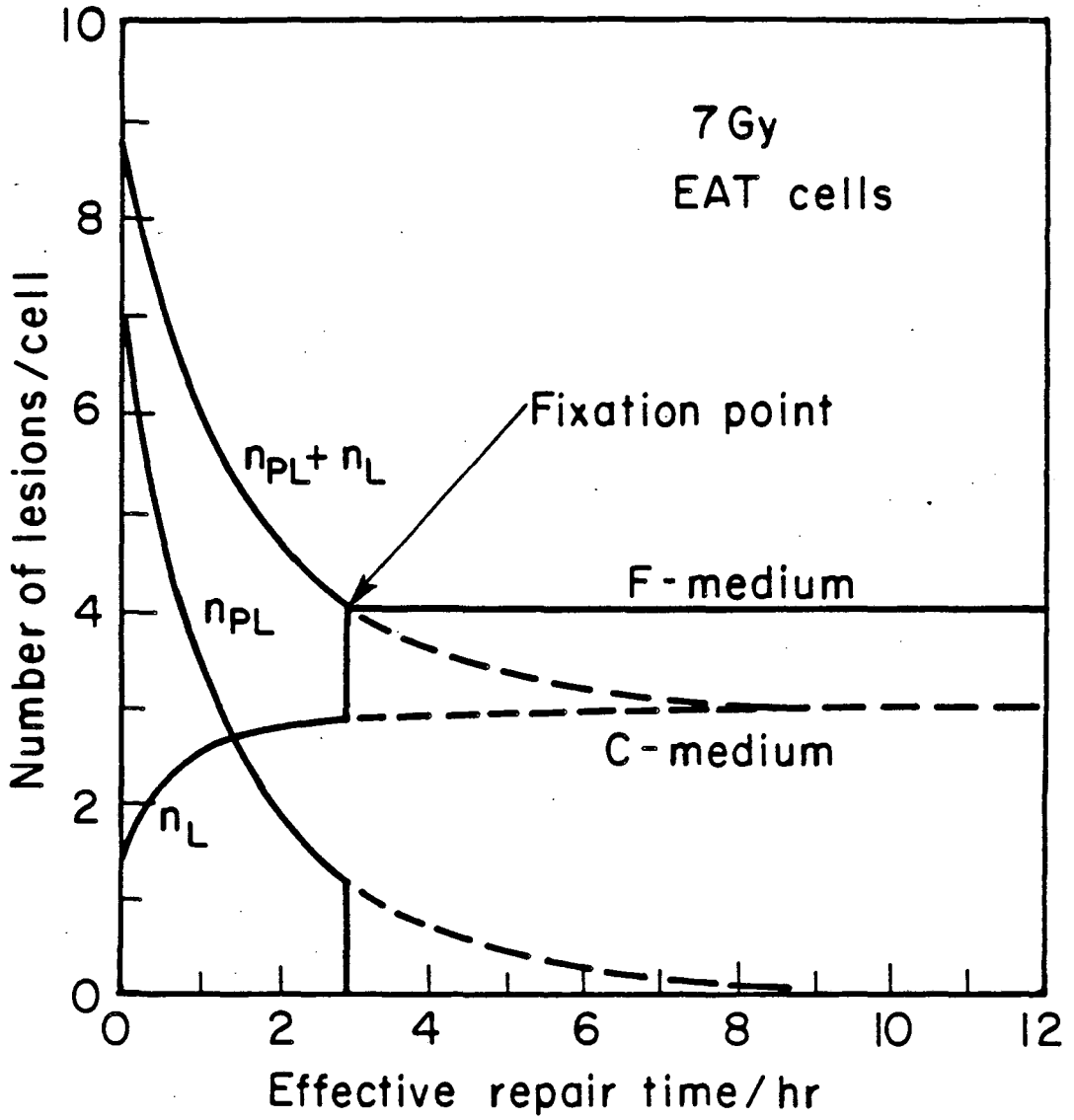
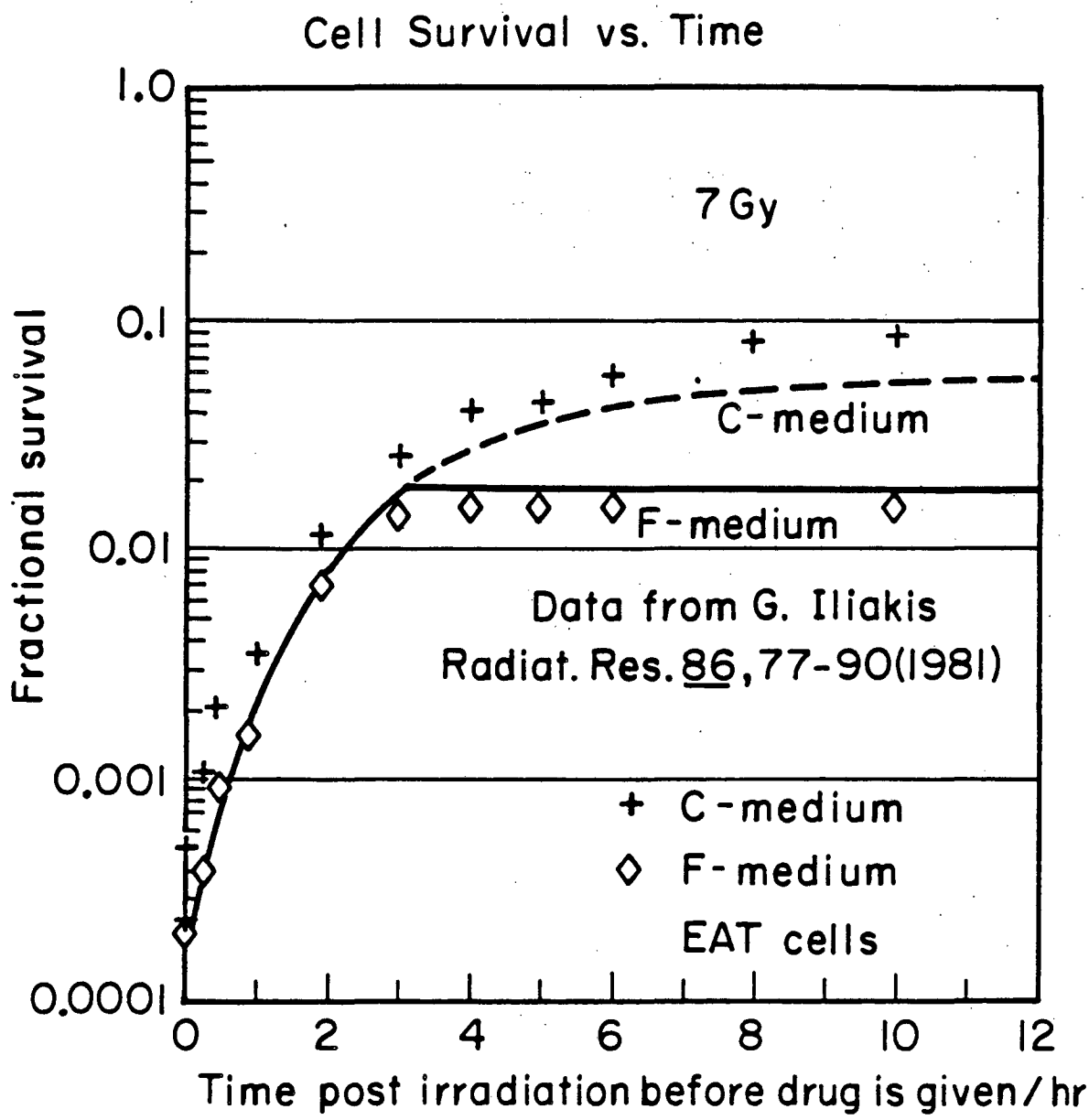

Dose Rate Effect 137Cs Gamma
C3H 10T½ Density Inhibited
55.8 Gy/hr
2.4
0.49
0.29
0.17
0.06
Exp'l. Data from Wells and Bedford
Radiat. Res. 94, 105-134 (1983)
RELATIVE SURVIVING FRACTION
DOSE (Gy)
XBL8311-4138

Figure 5.



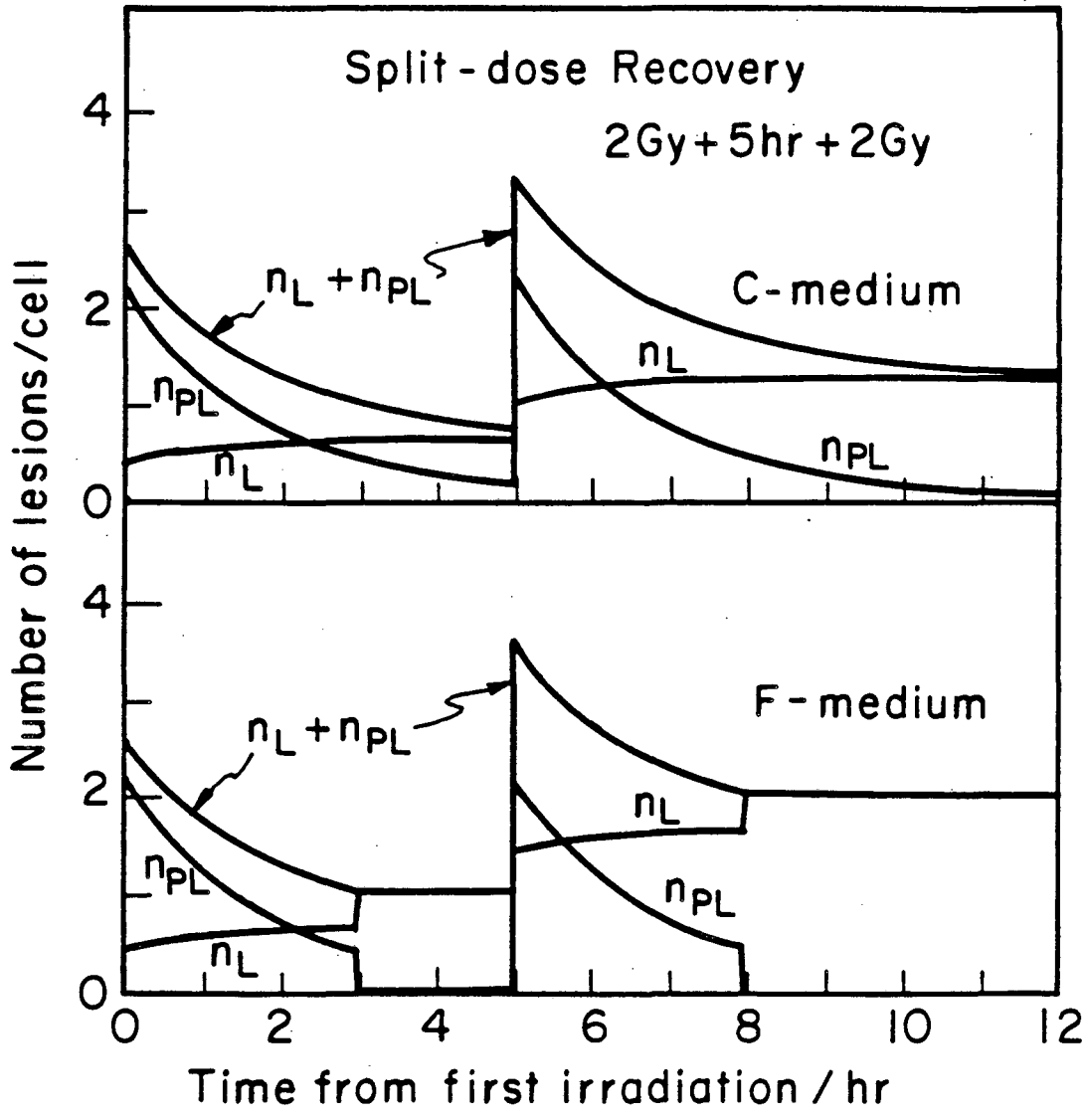
XBL 837-10638

Figure 6.



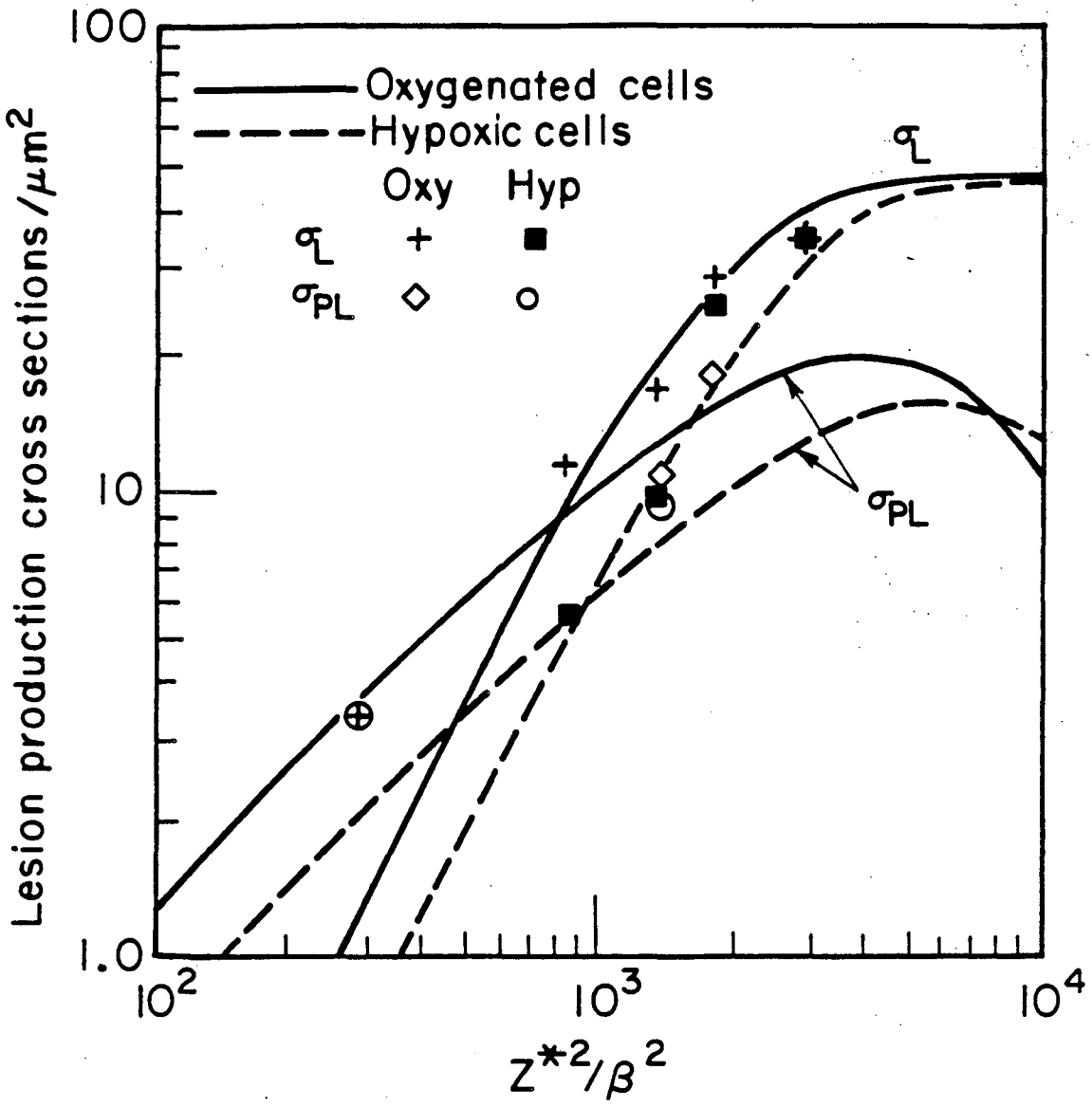
XBL 837-10639

Figure 7.



XBL 836-3802

Figure 8.



XBL 836-10340

Figure 9.

APPENDIX I

Equation (1) is seen to be of the general Riccati type, but can be directly integrated. We rewrite equation (1) and integrate:

$$\int_{o}^{n_{PL}} \frac{dn'_{PL}}{(\varepsilon_{2PL}\, n'^{2}_{PL} + \varepsilon_{PL}\, n'_{PL} - n_{PL}\dot{D})} = -\int_{o}^{t} dt' = -t \qquad \text{(I-1)}$$

If we let $\varepsilon_o = (4\varepsilon_{2PL}\, n_{PL}\dot{D} + \varepsilon^2_{PL})^{1/2}$, (I-2)

we can write the integral immediately from the tables:

$$\frac{1}{\varepsilon_o}\left[\ln \frac{2\varepsilon_{2PL}\, n_{PL} + \varepsilon_{PL} - \varepsilon_o}{2\varepsilon_{2PL}\, n_{PL} + \varepsilon_{PL} + \varepsilon_o} - \ln \frac{\varepsilon_{PL} - \varepsilon_o}{\varepsilon_{PL} + \varepsilon_o}\right] = -t \qquad \text{(I-3)}$$

Simplifying, remembering the definition of ε_o from equation (I-2), exponentiating each side and solving for n_{PL}, we obtain

$$n_{PL}(t) = \frac{2n_{PL}\dot{D}\,(1 - e^{-\varepsilon_o t})}{\varepsilon_o + \varepsilon_{PL} + (\varepsilon_o - \varepsilon_{PL})e^{-\varepsilon_o t}} \qquad \text{(I-4)}$$

Now to solve for $n_L(t)$, we can immediately write from equation (2):

$$n_L(t) = \int_{o}^{t} n_L\dot{D}\, dt' + \varepsilon_{2PL}\int_{o}^{t} n^2_{PL}(t')dt'$$

$$= n_L \dot{D} t + \varepsilon_{2PL} \int_0^t n_{PL}^2(t')dt' \qquad \text{(I-5)}$$

We change variables by letting:

$$x(t) = e^{-\varepsilon_0 t} \qquad \text{(I-6)}$$

Then $\frac{dx}{dt} = -\varepsilon_0 \, e^{-\varepsilon_0 t} = -\varepsilon_0 x$ and $dt/dx = -1/\varepsilon_0 x$

We can now rewrite equation (I-5):

$$n_L(t) = n_L \dot{D} t + \frac{\varepsilon_{2PL}}{\varepsilon_0} \int_x^1 \frac{n_{PL}^2(x')\, dx'}{x'} \qquad \text{(I-7)}$$

Looking only at the second term and, substituting equation (I-6) into equation (I-4), we have:

$$\int_x^1 \frac{n_{PL}^2(x')dx'}{x'} = 4(n_{PL}\dot{D})^2 \left[\int_x^1 \frac{dx'}{x'(a + b\, x')^2} - \int_x^1 \frac{2\, dx'}{(a + b\, x')^2} + \int_x^1 \frac{x'\, dx'}{(a + b\, x')^2} \right] \qquad \text{(I-8)}$$

where we have made the substitutions:

$$a = \varepsilon_0 + \varepsilon_{PL} \text{ and } b = \varepsilon_0 - \varepsilon_{PL} \qquad \text{(I-9)}$$

After again consulting the integral tables, and simplifying, we obtain:

$$n_L(t) = \eta_L \dot{D}t + \frac{4(\eta_{PL}\dot{D})^2 \varepsilon_{2PL}}{\varepsilon_o}\left[\frac{a^2-b^2}{a^2b^2}\ln\frac{a+b}{a+bx} - \frac{b^2\ln x}{a^2b^2} + \frac{(x-1)(a+b)}{ab(a+bx)}\right] \quad \text{(I-10)}$$

From the definitions of a and b [equation (I-9)] and ε_o [equation (I-2)], we can write:

$$a + b = 2\varepsilon_o$$

$$a^2 - b^2 = 4\varepsilon_o\varepsilon_{PL}$$

$$ab = 4\varepsilon_{2PL}\eta_{PL}\dot{D}$$

Then we can rewrite equation (I-10):

$$n_L(t) = \eta_L\dot{D}t + \frac{\varepsilon_{PL}}{\varepsilon_{2PL}}\ln\frac{2\varepsilon_o}{a+bx} + \frac{(\varepsilon_o-\varepsilon_{PL})^2 t}{4\varepsilon_{2PL}} + \frac{2\eta_{PL}\dot{D}(x-1)}{a+bx} \quad \text{(I-11)}$$

$$= \eta_L D + \frac{\varepsilon_{PL}}{\varepsilon_{2PL}}\left[\ln\frac{2\varepsilon_o}{\varepsilon_o+\varepsilon_{PL}+(\varepsilon_o-\varepsilon_{PL})e^{-\varepsilon_o t}}\right] + \frac{(\varepsilon_o-\varepsilon_{PL})^2 t}{4\varepsilon_{2PL}} - \frac{2\eta_{PL}\dot{D}(1-e^{-\varepsilon_o t})}{\varepsilon_o+\varepsilon_{PL}+(\varepsilon_o-\varepsilon_{PL})e^{-\varepsilon_o t}} \quad \text{(I-12)}$$

We note that the last term is just $\eta_{PL}(t)$ from equation (I-4).

APPENDIX II

The equations to be solved are equations (6) and (7) for times $t > T$:

$$\frac{dn_{PL}(t)}{dt} = -\varepsilon_{PL} n_{PL}(t) - \varepsilon_{2PL} n_{PL}^2(t) \qquad \text{(II-1)}$$

$$\frac{dn_2(t)}{dt} = -\varepsilon_{2PL} n_{PL}^2(t) \qquad \text{(II-2)}$$

with initial conditions:

$N_{PL} = n_{PL}(T)$, the solution from Appendix I for $t = T$

$N_L = n_L(T)$, the solution from Appendix I for $t = T$.

Working with equation (II-1), we can write:

$$\int_{N_{PL}}^{n_{PL}(t)} \frac{dn_{PL}}{\varepsilon_{PL} n_{PL} + \varepsilon_{2PL} n_{PL}^2} = -\int_T^t dt' = -(t-T) = -t_r \qquad \text{(II-3)}$$

where we have set $t_r = t-T$ = the time available for repair after the end of the exposure. The left side can be immediately integrated, using the tables, yielding:

$$1/\varepsilon_{PL} \quad \ln\left[\frac{\varepsilon_{PL} + \varepsilon_{2P} n_{PL}(t)}{n_{PL}(t)} - \ln \frac{\varepsilon_{PL} + \varepsilon_{2PL} N_{PL}}{N_{PL}}\right] = t_r \qquad \text{(II-4)}$$

Simplifying, we obtain:

$$\frac{[\varepsilon_{PL} + \varepsilon_{2PL} n_{PL}(t)]\, N_{PL}}{[\varepsilon_{PL} + \varepsilon_{2PL} N_{PL}]\, n_{PL}(t)} = e^{\varepsilon_{PL} t_r} \qquad \text{(II-5)}$$

Solving for $n_{PL}(t)$ yields:

$$n_{PL}(t) = \frac{N_{PL} e^{-\varepsilon_{PL} t_r}}{1 + (N_{PL}/\varepsilon)(1-e^{-\varepsilon_{PL} t_r})} \qquad \text{(II-6)}$$

where we have set $\varepsilon = \varepsilon_{PL}/\varepsilon_{2PL}$ and $t_r = t-T$

We now solve for $n_L(t)$:

$$\int_{n_L(T)}^{n_L(t)} dn_L = \varepsilon_{2PL} \int_T^t n_{PL}^2 (t')dt' = \varepsilon_{2PL} \int_0^{t_r} n_{PL}^2 (t_r')dt_r' \qquad \text{(II-7)}$$

Changing the variable of integration to $t_r' = t' - T$:

$$n_L(t) = n_L(T) + \varepsilon_{2PL} \int_0^{t_r} \left[\frac{N_{PL} e^{-\varepsilon_{PL} t_r'}}{1 + (N_{PL}/\varepsilon)(1 - e^{-\varepsilon_{PL} t_r'})} \right]^2 dt_r' \qquad \text{(II-8)}$$

$$= n_L(T) + \varepsilon_{2PL} N_{PL} \int_0^{t_r} \frac{e^{-2\varepsilon_{PL} t_r'} dt_r'}{\left[1 + (N_{PL}/\varepsilon)(1 - e^{-\varepsilon_{PL} t_r'})\right]^2} \qquad \text{(II-9)}$$

By introducing a new variable of integration $x' = e^{-\varepsilon_{PL} t_r'}$, $dt_r' = (-dx')/(\varepsilon_{PL} x')$, and two new constants $a = (1 + N_{PL}/\varepsilon)$ and $b = -N_{PL}/\varepsilon$, the above integral can be looked up in the integral tables yielding:

$$-\varepsilon \left[\ln (a+bx) + \frac{a}{a + bx} - \ln (a+b) - \frac{a}{a + b} \right] \qquad \text{(II-10)}$$

Substituting back for t_r, N_{PL}, and ε, we obtain for $n_L(t_r)$:

$$n_L(t_r) = n_L(T) - \varepsilon \ln \left[1 + N_{PL}/\varepsilon \, (1-e^{-\varepsilon_{PL} t_r}) \right]$$

$$+ \frac{N_{PL} \, (1 + N_{PL}/\varepsilon) \, (1 - e^{-\varepsilon_{PL} t_r})}{1 + N_{PL}/\varepsilon \, (1 - e^{-\varepsilon_{PL} t_r})} \qquad \text{(II-11)}$$

APPENDIX III

We will show here that when $\dot{D} << \varepsilon_{PL}/\eta_{PL}$ and $\varepsilon > 2$, the survival curve becomes an exponential function of dose. We expand ε_0 as follows:

$$\varepsilon_0 = (\varepsilon_{PL}^2 + 4\eta_{PL}\dot{D}\varepsilon_{2PL})^{1/2} = \varepsilon_{PL}\left(1 + \frac{4\eta_{PL}\dot{D}\varepsilon_{2PL}}{\varepsilon_{PL}^2}\right)^{1/2}$$

$$= \varepsilon_{PL}\left(1 + \frac{4\eta_{PL}\dot{D}}{\varepsilon\ \varepsilon_{PL}}\right)^{1/2}$$

$$= \varepsilon_{PL}\left(1 + \frac{2\eta_{PL}\dot{D}}{\varepsilon\ \varepsilon_{PL}} + \ldots\right) \qquad \text{(III-1)}$$

Now if $1 >> 2\eta_{PL}\dot{D}/(\varepsilon\ \varepsilon_{PL})$, $\varepsilon_0 = \varepsilon_{PL}$.

This restriction then becomes

$$\dot{D} << \varepsilon\ \varepsilon_{PL}/(2\eta_{PL}) \qquad \text{(III-2)}$$

So if the additional restriction of $\varepsilon > 2$ is true, then

$$\dot{D} << \varepsilon_{PL}/\eta_{PL} \qquad \text{(III-3)}$$

satisfies the above inequality and $\varepsilon_0 = \varepsilon_{PL}$.

An inspection of equations (3) and (5) shows that, if this is the case,

$$n_{PL}(t) = \frac{\eta_{PL}\dot{D}\,(1 - e^{-\varepsilon_{PL}t})}{\varepsilon_{PL}} \qquad \text{(III-4)}$$

$$n_L(t) = \eta_L D - n_{PL}(t) \qquad \text{(III-5)}$$

Therefore, at the end of the exposure,

$$n_{TOT}(T) = n_{PL}(T) + n_L(T) = \eta_L D = N_{TOT} \qquad \text{(III-6)}$$

The survival expression, equation (12) becomes:

$$S = e^{-\eta_L D}\left[1 + \frac{\eta_{PL}\dot{D}\,(1 - e^{-\varepsilon_{PL}T})}{\varepsilon_{PL}\,\varepsilon}\,(1 - e^{-\varepsilon_{PL}(t'-T)})\right]^{\varepsilon} \qquad \text{(III-7)}$$

Since both expressions within the parentheses are always less than unity, the second term in brackets can be neglected when

$$(\eta_{PL}\dot{D})/(\varepsilon_{PL}\,\varepsilon) \ll 1 \quad \text{or} \quad \dot{D} \ll \varepsilon_{PL}\,\varepsilon/\eta_{PL} \qquad \text{(III-8)}$$

This will certainly be the case when $\dot{D} \ll \varepsilon_{PL}/\eta_{PL}$ and $\varepsilon > 2$, the two restrictions assumed above. Therefore, the survival expression reduces to:

$$S = e^{-\eta_L D} \qquad \text{(III-9)}$$

when $\dot{D} \ll \varepsilon_{PL}/\eta_{PL}$ and $\varepsilon > 2$.

APPENDIX IV

The high dose rate restriction is given as:

$$\dot{D} \gg \varepsilon_{PL}^2/(4\eta_{PL}\varepsilon_{2PL}).$$

We will show this leads to equation (16) with an additional restriction on the exposure time, T.

We start with the two general equations for $n_{PL}(T)$ and $n_{TOT}(T)$:

$$n_{PL}(T) = 2\eta_{PL}\dot{D}(1-e^{-\varepsilon_0 T})/[(\varepsilon_0 + \varepsilon_{PL}) + (\varepsilon_0 - \varepsilon_{PL})e^{-\varepsilon_0 T}] \qquad \text{(IV-1)}$$

$$n_{TOT}(T) = n_{PL}(T) + n_L(T)$$

$$= \eta_L D + \frac{\varepsilon_{PL}}{\varepsilon_{2PL}} \ln \frac{2\varepsilon_0}{\varepsilon_0 + \varepsilon_{PL} + (\varepsilon_0 - \varepsilon_{PL})e^{-\varepsilon_0 T}}$$

$$+ \frac{(\varepsilon_0 - \varepsilon_{PL})^2 T}{4\varepsilon_{2PL}} \qquad \text{(IV-2)}$$

First we use the restriction of short irradiation times (i.e., $T \ll 2/\varepsilon_0$) to make the approximation $e^{-\varepsilon_0 T} = 1 - \varepsilon_0 T$ and $1 - e^{-\varepsilon_0 T} = \varepsilon_0 T$. Then:

$$n_{PL}(T) = \frac{2 n_{PL} \dot{D} \varepsilon_0 T}{\varepsilon_0 + \varepsilon_{PL} + [(\varepsilon_0 - \varepsilon_{PL})(1-\varepsilon_0 T)]}$$

$$= \frac{n_{PL} \dot{D} T}{1 - [1/2(\varepsilon_0 - \varepsilon_{PL})T]} = n_{PL} \dot{D} T = n_{PL} D \qquad \text{(IV-3)}$$

We have neglected $1/2(\varepsilon_0 - \varepsilon_{PL})T$ in the denominator by invoking the short irradiation time restriction. We note here that if $T \ll 2/\varepsilon_0$, then $T \ll 2/(\varepsilon_0 - \varepsilon_{PL})$ since ε_{PL} is always positive and less than or equal to ε_0.

Now from equation (IV-2), we see that the second term on the right can be simplified by expanding the exponential in the denominator:

$$\varepsilon \ln \frac{2\varepsilon_0}{\varepsilon_0 + \varepsilon_{PL} + (\varepsilon_0 - \varepsilon_{PL})e^{-\varepsilon_0 T}}$$

$$= \varepsilon \ln \frac{2\varepsilon_0}{\varepsilon_0 + \varepsilon_{PL} + (\varepsilon_0 - \varepsilon_{PL})(1-\varepsilon_0 T)}$$

$$= \varepsilon \ln \frac{1}{1-[1/2\ (\varepsilon_0 - \varepsilon_{PL})\ T]}$$

$$= -\varepsilon \ln \left\{ 1-[1/2(\varepsilon_0 - \varepsilon_{PL})T] \right\}$$

We expand the log in a series expansion requiring now that

$T << \dfrac{4}{(\varepsilon_o - \varepsilon_{PL})}$; remembering the definition of ε, we obtain

$$= -\varepsilon(-1/2[\varepsilon_o - \varepsilon_{PL})T) = \frac{\varepsilon_{PL}}{2\varepsilon_{2PL}}(\varepsilon_o - \varepsilon_{PL})T = \frac{\varepsilon_{PL}\varepsilon_o T}{2\varepsilon_{2PL}} - \frac{\varepsilon_{PL}^2 T}{2\varepsilon_{2PL}} \qquad \text{(IV-4)}$$

The third term on the right of equation (IV-2) can be written:

$$\frac{(\varepsilon_o^2 - 2\varepsilon_o\,\varepsilon_{PL} + \varepsilon_{PL}^2)T}{4\varepsilon_{2PL}} = \frac{\varepsilon_o^2 T}{4\varepsilon_{2PL}} - \frac{\varepsilon_o\,\varepsilon_{PL} T}{2\varepsilon_{2PL}} + \frac{\varepsilon_{PL}^2 T}{4\varepsilon_{2PL}} \qquad \text{(IV-5)}$$

Now, upon adding this term to the one derived in equation (IV-4), we note a cancellation yielding:

$$-\frac{\varepsilon_{PL}^2 T}{2\varepsilon_{2PL}} + \frac{\varepsilon_o^2 T}{4\varepsilon_{2PL}} + \frac{\varepsilon_{PL}^2 T}{4\varepsilon_{2PL}} = \frac{(\varepsilon_o^2 - \varepsilon_{PL}^2)T}{4\varepsilon_{2PL}}$$

Equation (IV-2) becomes

$$\eta_{TOT}(T) = \eta_L^D + \frac{(\varepsilon_o^2 - \varepsilon_{PL}^2)T}{4\varepsilon_{2PL}} \qquad \text{(IV-6)}$$

Now, if we require $\varepsilon_o^2 >> \varepsilon_{PL}^2$, we can write

$$\eta_{TOT}(T) = \eta_L^D + \frac{\varepsilon_o^2 T}{4\varepsilon_{2PL}} \qquad \text{(IV-7)}$$

But from the definition of ε_0 (equation (4)), we see

$$\varepsilon_0^2 = 4\eta_{PL}\dot{D}\varepsilon_{2PL} + \varepsilon_{PL}^2$$

and if $\varepsilon_0^2 >> \varepsilon_{PL}^2$,

$$4\eta_{PL}\dot{D}\varepsilon_{2PL} + \varepsilon_{PL}^2 >> \varepsilon_{PL}^2$$

$$\frac{4\eta_{PL}\dot{D}\varepsilon_{2PL}}{\varepsilon_{PL}^2} + 1 >> 1$$

So $$\frac{4\eta_{PL}\dot{D}\varepsilon_{2PL}}{\varepsilon_{PL}^2} >> 1 \qquad \text{(IV-8)}$$

and the restriction on the dose rate, $\dot{D}$, becomes

$$\dot{D} >> \frac{\varepsilon_{PL}^2}{4\eta_{PL}\varepsilon_{2PL}} \quad \text{(high dose rate)} \qquad \text{(IV-9)}$$

Also, from the above inequality, (IV-8),

$$4\eta_{PL}\dot{D}\varepsilon_{2PL} >> \varepsilon_{PL}^2$$

and we see that ε_0^2 reduces to

$$\varepsilon_0^2 = 4\eta_{PL}\dot{D}\varepsilon_{2PL}.$$

Now substituting this expression for ε_0^2 into equation (IV-7), we obtain

$$n_{TOT}(T) = \eta_L D + \frac{4\eta_{PL}\dot{D}\ \varepsilon_{2PL}T}{4\varepsilon_{2PL}}$$

$$= \eta_L D + \eta_{PL}\dot{D}T = \eta_L D + \eta_{PL}D. \qquad \text{(IV-10)}$$

Therefore, using this expression for N_{TOT} and equation (IV-3) for N_{PL} in equation (12), we arrive at equation (16).

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