Title
THE LETHAL, POTENTIALLY LETHAL LESION MODEL

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MAJOR HYPOTHESES OF THE LETHAL, POTENTIALLY LETHAL LESION MODEL

1. Two types of long-lived lesions (designated "lethal" and "potentially lethal") are relevant to cell killing, and remain in the cell after the irradiation of a cell population.

2. The lethal lesions cannot be repaired and result in the eventual death of the cell or its progeny. They are created by the fast interaction, or maybe just the close proximity, of two or more "sublesions" formed by a single charged particle track within a critical distance, perhaps on the order of tens of nanometers, depending on the chemical environment.

3. Sublesions are caused by "clusters" of ionizations (perhaps 6 to 10 are necessary within a distance of 2 to 3 nanometers). These sublesions are tentatively being assumed to lead to double-strand breaks in DNA.

4. Isolated sublesions can lead to potentially lethal lesions. This process is modified by chemical restitution processes depending on radical concentration and diffusion, oxygen concentration, and the presence of sulfhydral compounds within the cell nucleus.

5. If given sufficient time (for example, in a "delayed plating" experiment), the potentially lethal lesions are either correctly repaired or they interact with each other ("misrepair") producing a lesion that is lethal to the cell or its progeny. This process does not depend on the initial proximity of the two lesions, but instead depends on the square of the lesion concentration.

6. If an experimental procedure interrupts the repair process (for example, trypsinization and the initiation of the cell proliferation cycle or the addition of a repair inhibiting drug), the potentially lethal lesions can be "fixed," i.e., made lethal.

A rough schematic picture of the early time course of events leading to cell lethality is shown in Figure 1. A diagramatic sketch of the model is given in Figure 2; it has a starting point (in the biological time frame) identical to one version of the cybernetic model as developed by Pohlit (1981).

Assuming a Poisson distribution of the number of lesions/cell, the above assumptions lead to a survival expression:

$$S = \exp\left[-(\eta_{AC} + \eta_{AB})D\right]\left[1 + \frac{\eta_{AB}D}{\varepsilon}\left(1 - \exp(-\varepsilon_{BA}t)\right)\right]^{\varepsilon} \qquad (1)$$

where η_{AC} = production rate per unit dose of lethal lesions

η_{AB} = production rate per unit dose of potentially lethal lesions

ε = ratio of correct repair to misrepair rates = $\varepsilon_{BA}/\varepsilon_{BC}$

ε_{BA} = correct repair rate

t = effective time for repair

VARIATION OF RADIATION QUALITY FOR "TRACK SEGMENT" EXPERIMENTS

We assume that the number of lesions produced is proportional to particle fluence; i.e., $\eta D = \sigma\Phi$ with Φ = fluence.

Noting that $D = L\,\Phi$ with L = the particle LET_{∞}, we can rewrite the above survival equation:

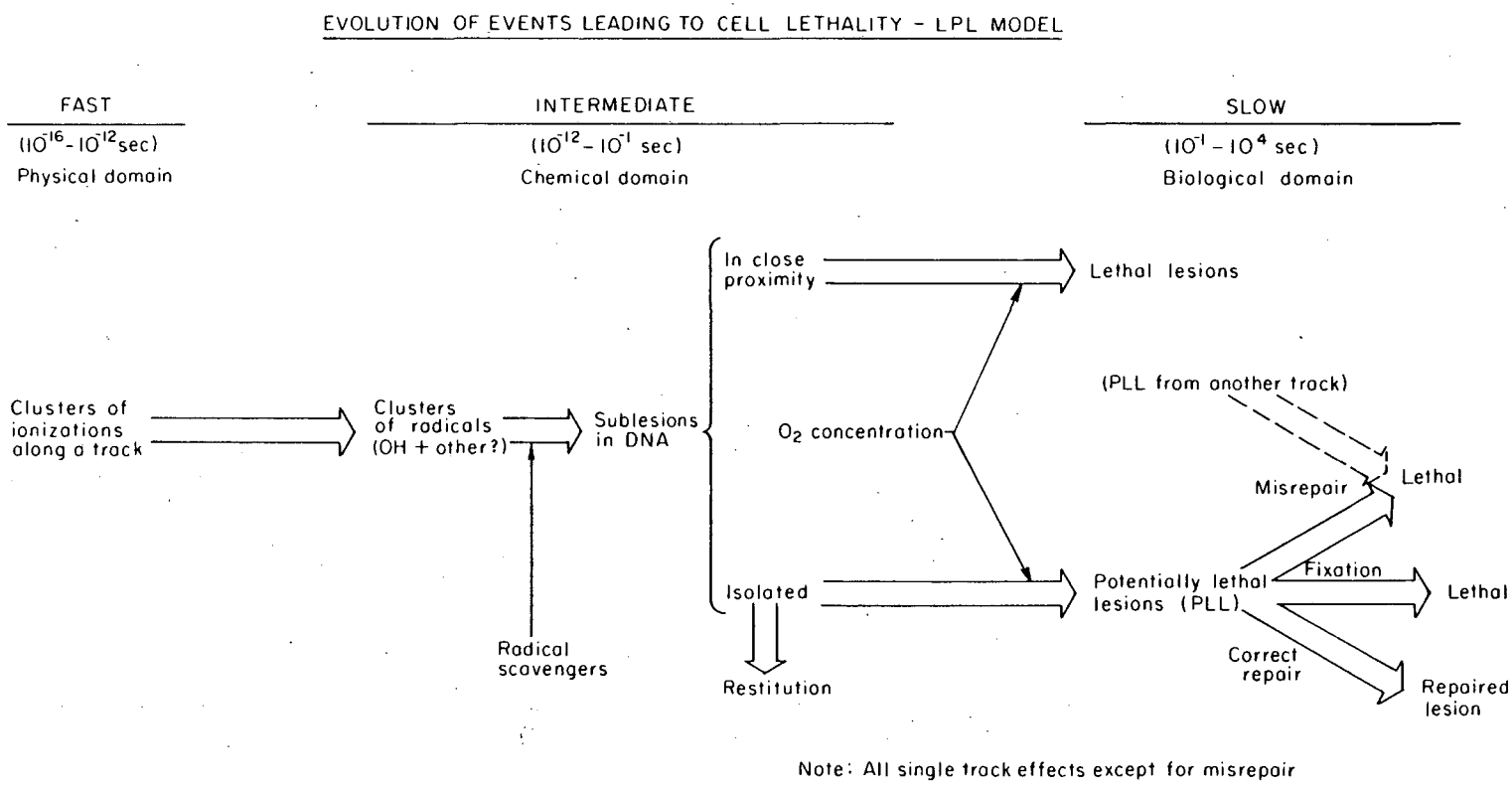

Figure 1. Evolution of important events leading to cell lethality in the LPL model. Clusters of ionizations (physical domain) lead to clusters of radicals which in turn lead to sublesions in DNA (chemical domain). If these are in close proximity, they lead to lethal (irrepairable) lesions. If they are isolated, they can, if not restituted, lead to potentially lethal (repairable) lesions. The latter (biological domain) can either interact to form a lethal lesion (misrepair), can be "fixed" at some point in the cell cycle, or can be correctly repaired. (XBL 837-10634)

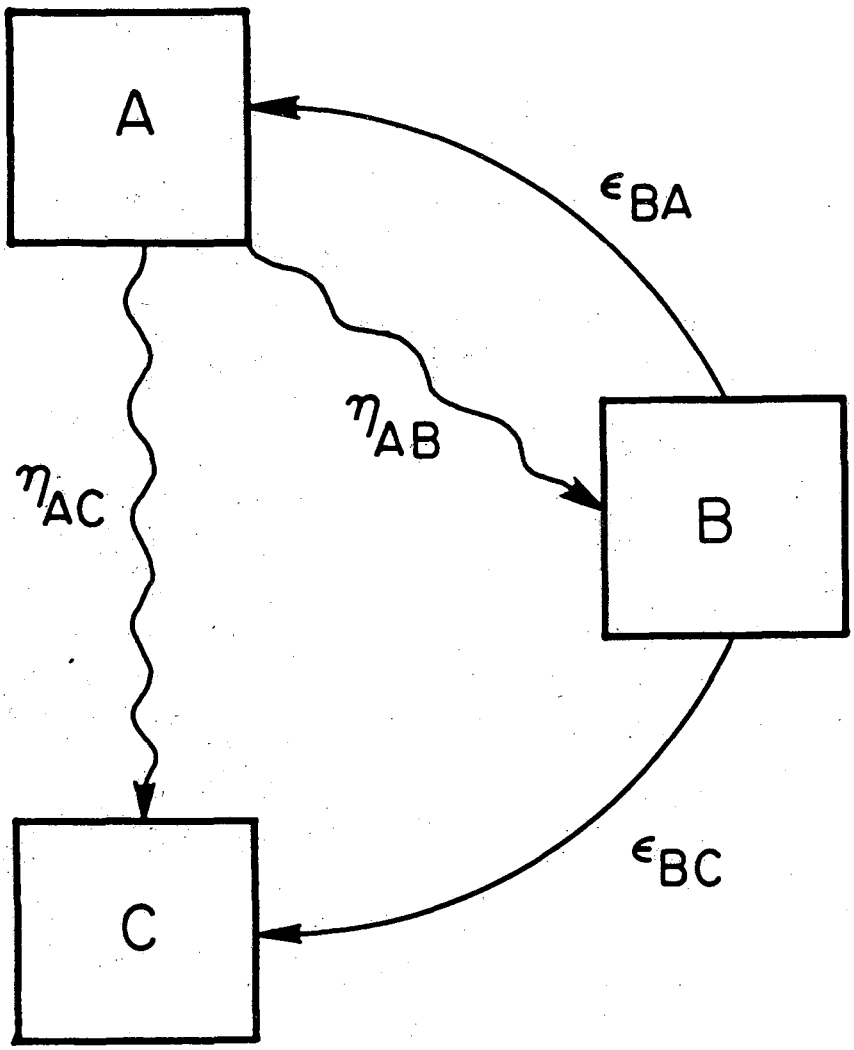

Figure 2. Schematic representation of the formation of lethal (C) and potentially lethal (B) lesions with η_{AB} and η_{AC} the rate per unit of absorbed dose for the production of B and C lesions, respectively. The potentially lethal lesions can either repair correctly with rate ϵ_{BA} or misrepair with rate ϵ_{BC}. (XBL 829-4114)

$$S = \exp\left[-\frac{1}{L}(\sigma_{AC} + \sigma_{AB})D\right]\left[1 + \frac{\sigma_{AB}D}{L\varepsilon}(1 - \exp(-\varepsilon_{BA}t))\right]^{\varepsilon} \qquad (2)$$

The cross sections, σ_{AC} and σ_{AB} are the probabilities per unit fluence to produce the lethal and potentially lethal lesions, respectively.

ASSUMPTIONS FOR THE CROSS SECTIONS

1. Sublesions are distributed along each track in a Poisson distribution. (We define λ = mean distance between sublesions.)

2. The distance of the sublesions from the trajectory is small compared to their separation.

3. There are on the average n critical regions (targets) of length x_o along the track through the cell nucleus.

4. A lethal lesion is caused by two or more sublesions occurring within a critical region of length x_o along the track.

5. A potentially lethal lesion can arise from isolated sublesions.

6. The cell nuclei have a radiobiologically effective cross section σ_o on the average presented to the particle beam.

Then:

P_o = $\exp(-x_o/\lambda)$ = probability of finding no sublesions in distance x_o along the track;

$P_1 = x_o/\lambda \exp(-x_o/\lambda)$ = probability of finding one and only one sublesion in x_o;

$P_{\geq 2} = 1-P_o-P_1$ = probability of finding two or more sublesions in x_o.

The probability of finding at least one lethal lesion along a track within a cell nucleus is:

$$P_{lethal} = 1 - (1 - P_{\geq 2})^n \tag{3}$$

and the cross section for lethal lesion production (probability per unit fluence) is:

$$\sigma_{AC} = \sigma_o[1-(1-P_{\geq 2})^n] = \sigma_o \{1-[\exp(-x_o/\lambda)(1 + x_o/\lambda)]^n\} \tag{4}$$

The probability of finding an isolated sublesion along a track, $1-(1-P_1)^n$, leads to the equation for the production cross section for potentially lethal lesions:

$$\sigma_{AB} = F_{PL}\, \sigma_o\{1 - [1 - x_o/\lambda \exp(-x_o/\lambda)]^n\} \tag{5}$$

Experimental data indicate that every isolated sublesion does not lead to a potentially lethal lesion, i.e., chemical restitution processes play a role in modifying the production of potentially lethal lesions. The probability for chemical restitution is given by F_{PL} in the above equation. It depends on the chemical constitution in the cell nucleus such as the presence of sulfhydrals and oxygen.

We note that for large λ, i.e., at low LET:

$$\sigma_{AC} \simeq n\sigma_o \cdot (x_o^2/2\lambda^2) \text{ and } \sigma_{AB} \simeq nF_{PL}\,\sigma_o(x_o/\lambda). \quad (6)$$

Lacking physical data on geometrical distributions of ionizations around and along particle tracks within the cell nucleus, we make one further assumption, valid only in a limited range of particle effective charge, Z*, and velocity, βc:

$$x_o/\lambda = k(Z^*/\beta)^2 \quad (7)$$

i.e., the mean number of clusters in a length x_o along the track is proportional to the square of the ratio of the effective charge and β. Then:

$$\sigma_{AC}(Z^*,\beta) = \sigma_o\{1 - [\exp(-kZ^{*2}/\beta^2)\ (1 + kZ^{*2}/\beta^2)]^n\} \quad (8)$$

$$\sigma_{AB}(Z^*,\beta) = \sigma_o\, F_{PL}\{1 - [1 - (kZ^{*2}/\beta^2)\ \exp(-kZ^{*2}/\beta^2)]^n\} \quad (9)$$

As an example, Figure 3 shows σ_{AC} and σ_{AB} 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} = 0.3 (oxygenated cells), F_{PL} = 0.15 (hypoxic cells)

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

A comparison is made with best fit values of σ_{AC} and σ_{AB} obtained from the survival of T-1 human kidney cells irradiated with alpha particles (Barendsen et al., 1966).

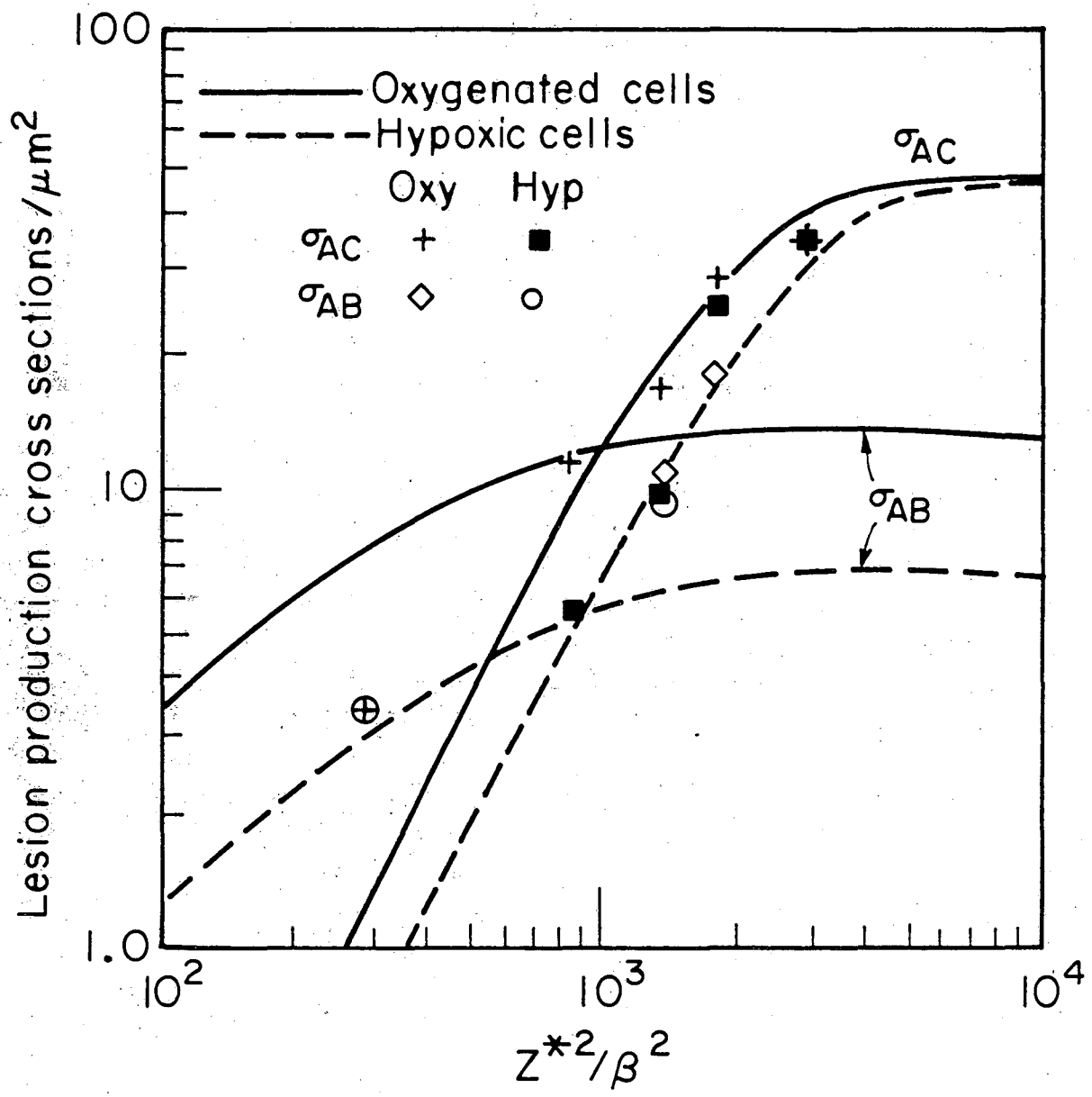

Figure 3. Lesion production cross sections, σ_{AC} and σ_{AB}, as a function of Z^{*2}/β^2 for oxygenated (solid line) or hypoxic (dashed line) cells. Data points were obtained from best fits to cell survival curves obtained with human kidney T-1 cells irradiated with alpha particles and deuterons of various velocities (Barendsen et al., 1966). Values of the parameters used to calculate the curves are given in the text. (XBL 836-10340)

SUBLETHAL AND POTENTIALLY LETHAL DAMAGE IN THE LPL MODEL

Equation (1) can be written:

$$S = \exp\left[-n_B(n_{OB},t) - n_C(n_{OC},t)\right] \tag{10}$$

$$\text{with: } n_B(n_{OB},t) = n_{OB}\ \exp(-\varepsilon_{BA}t)/\left[1 + \frac{n_{OB}}{\varepsilon}\ (1 - \exp(-\varepsilon_{BA}t)\right] \tag{11}$$

where n_{OB} = the initial number of potentially lethal lesions produced by a dose, D, and

$$n_C(n_{OC},t) = n_{OC} + n_{OB} - n_B(n_{OB},t) - \varepsilon \ln\left[1 + \frac{n_{OB}}{\varepsilon} \cdot (1-\exp(-\varepsilon_{BA}t)\right] \tag{12}$$

where n_{OC} = the initial number of lethal lesions produced by an absorbed dose, D.

For simplicity, we will consider only stationary (plateau) phase cells. There is experimental evidence that in an immediate plating experiment, a considerable time period, t_o, (about 3 hours) can be available for repair after plating. After this period, the remaining potentially lethal lesions are assumed to be "fixed," perhaps by their passing through a "fixation" point in the cell cycle. Thus, the survival equation for an immediate plating experiment is:

$$S = \exp\left[-n_B(n_{OB},t_o) - n_C(n_{OC},t_o)\right] \tag{13}$$

This reduces to:

$$S = \exp\ (-n_{OB}-n_{OC})\ \left[1 + \frac{n_{OB}}{\varepsilon}\ (1-\exp(-\varepsilon_{BA}t_o)\right]^{\varepsilon} \qquad (14)$$

Similarly, for a <u>delayed plating</u> experiment, an "infinite" time for repair is allowed and the survival expression reduces to:

$$S = \exp\ (-n_{OB}-n_{OC})\left[1 + \frac{n_{OB}}{\varepsilon}\right]^{\varepsilon} \qquad (15)$$

A conventional delayed plating (PLD) experiment is shown in Figure 4. Here all the time points really include 3 hours of repair (not measureable) "on the plate." Note: All examples use the following values of the model parameters: $\eta_{AC} = 0.2\ Gy^{-1}$; $\eta_{AB} = 1.1\ Gy^{-1}$; $\varepsilon = 10$; and $\varepsilon_{BA} = 0.5\ hr^{-1}$.

For an experiment in which it is assumed that, at some time after the experiment, all repair is stopped and damage is fixed (e.g., with the use of β-araA), we can write the survival as a function of repair time, t_{rep}:

$$S(D,t_{rep}) = \exp\ \left[-n_B(\eta_{AB}D,t_{rep}) - n_C(\eta_{AC}D,t_{rep})\right] \qquad (16)$$

where we have again assumed that the initial number of each kind of lesion is proportional to the absorbed dose, D; $n_{OB} = \eta_{AB}D$ and $n_{OC} = \eta_{AC}D$.

Figure 5 shows the time course of the two different kinds of lesions and their total. Figure 6 shows the variation with time of the survival in such an experiment. If repair continues to occur <u>after</u> the repair inhibitor is removed and growth medium is added, t_{rep} must include the additional repair time, t_o.

A calculation for Ehrlich ascites tumor (EAT) cells in vitro is made at 7 Gy for fresh and conditioned media and compared in Figures 7 and 8. Figure 7 shows the time course of the lesions in each medium, and Figure 8 shows the

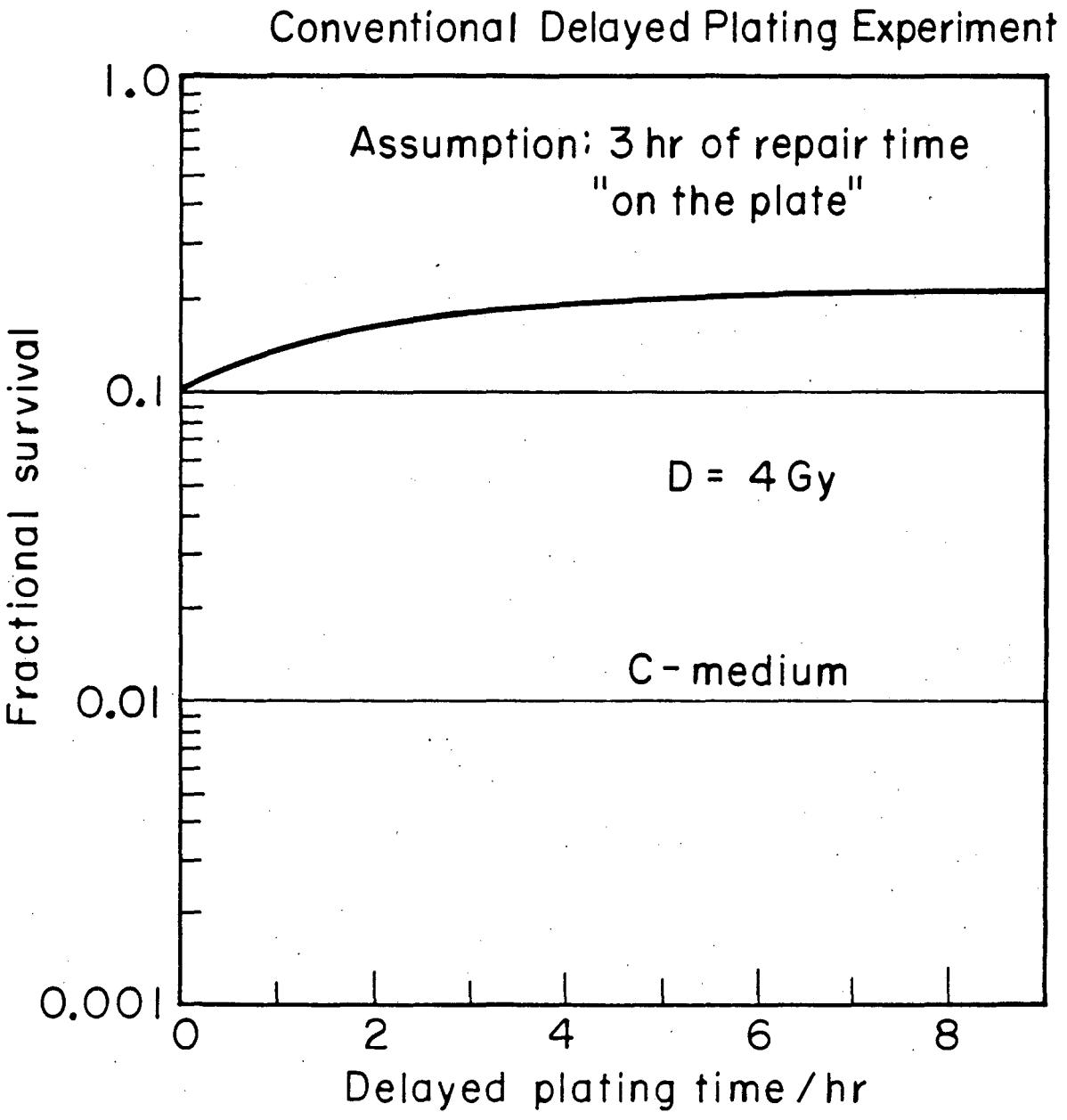

Figure 4. Cell survival as a function of delayed plating time in "conditioned medium" after an absorbed dose of 4 Gy, assuming 3 hours of repair occurs in the petri dish after plating. This may represent the situation in many conventional experiments measuring the repair of PLD. (XBL 837-10635)

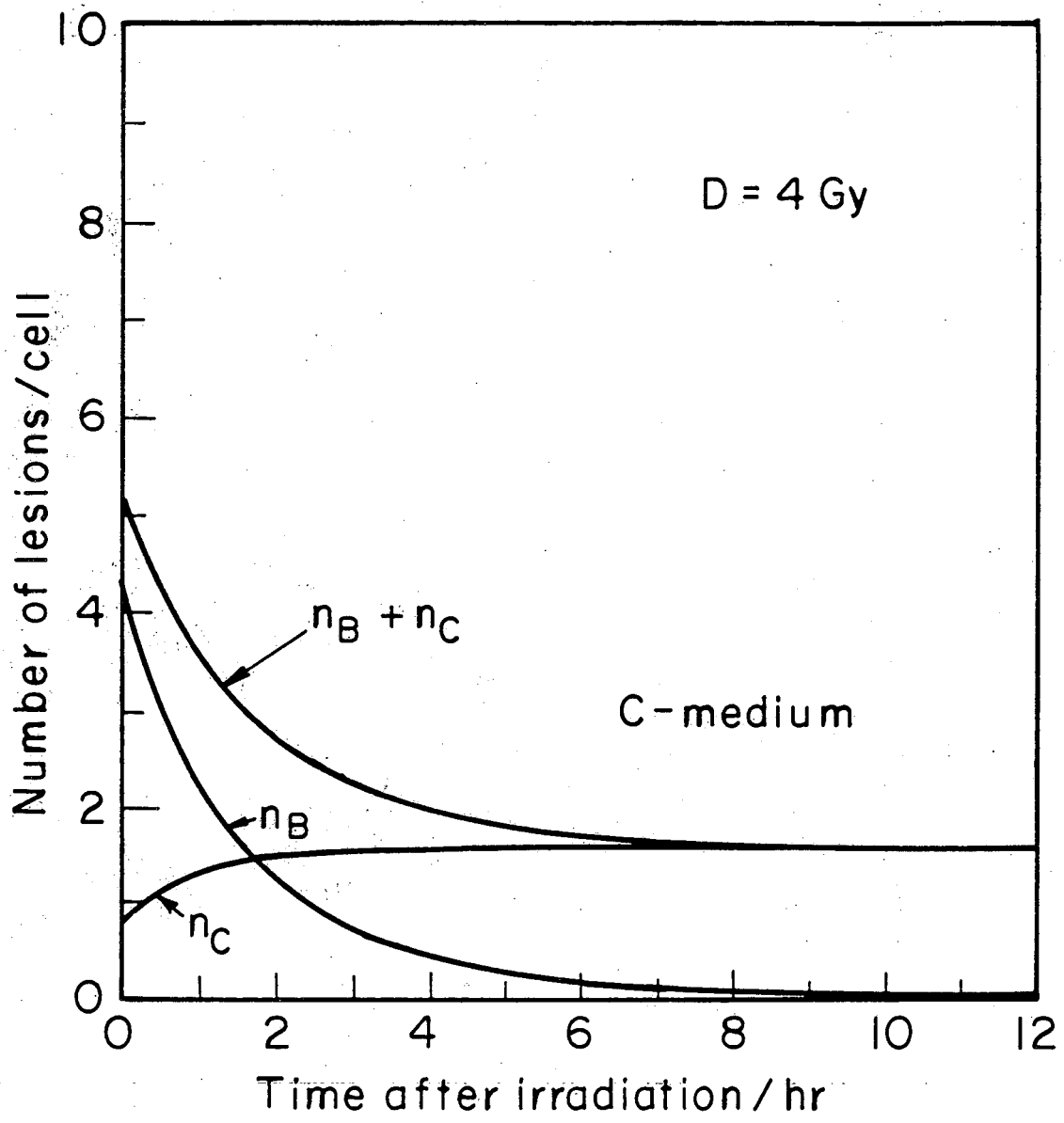

Figure 5. The time course of the mean numbers of lethal (n_C) and potentially lethal (n_B) lesions in "conditioned" medium as calculated from Equations (11) and (12) for an absorbed dose of 4 Gy. The increase of n_C with time indicates the occurrance of misrepair. (XBL 837-10636)

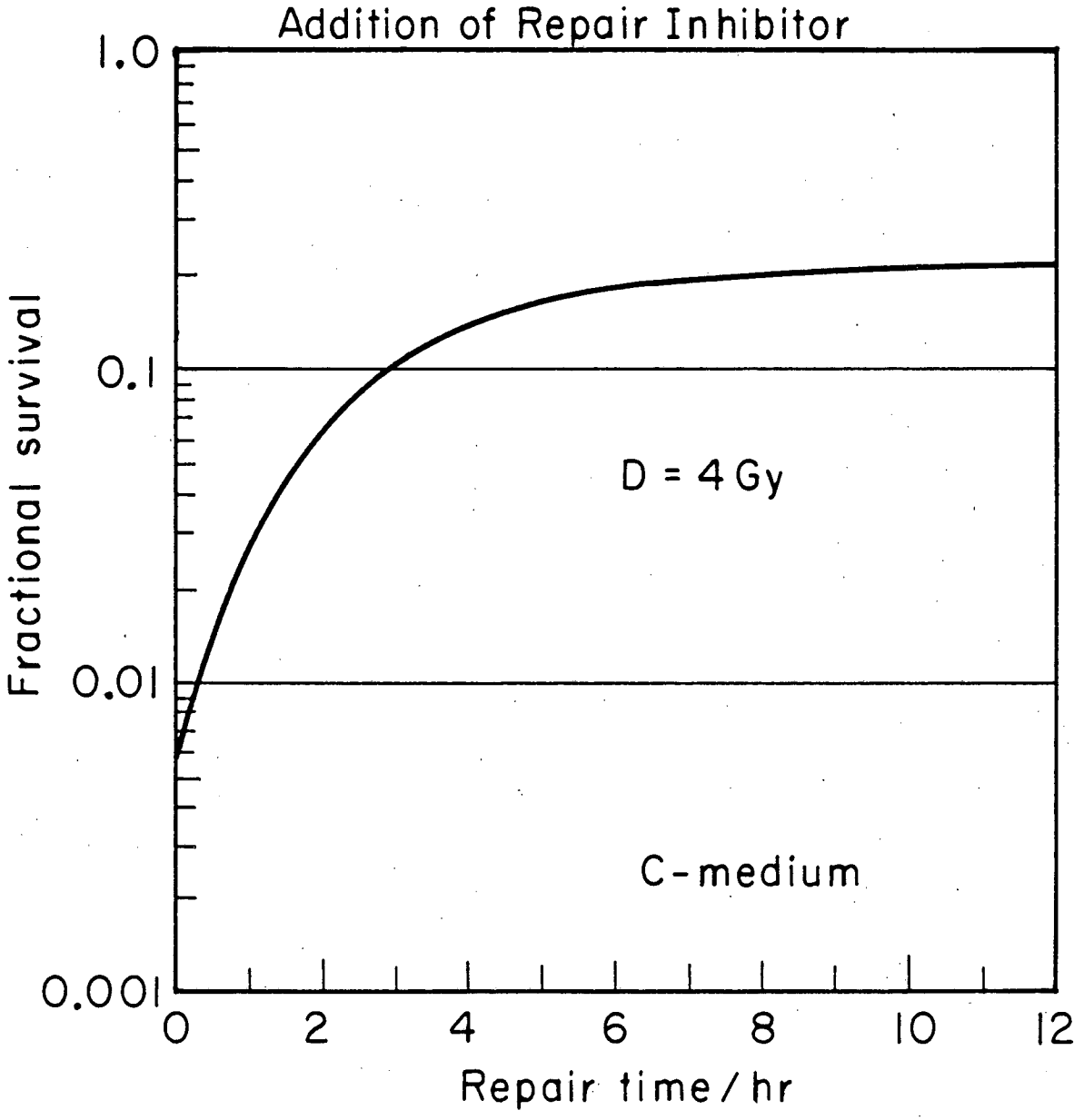

Figure 6. The time course of the cell survival in "conditioned" medium for an absorbed dose of 4 Gy, and corresponding directly to the lesion production, repair, and misrepair shown in Figure 5. This would be the result of an ideal repair inhibitor experiment. (XBL 837-10637)

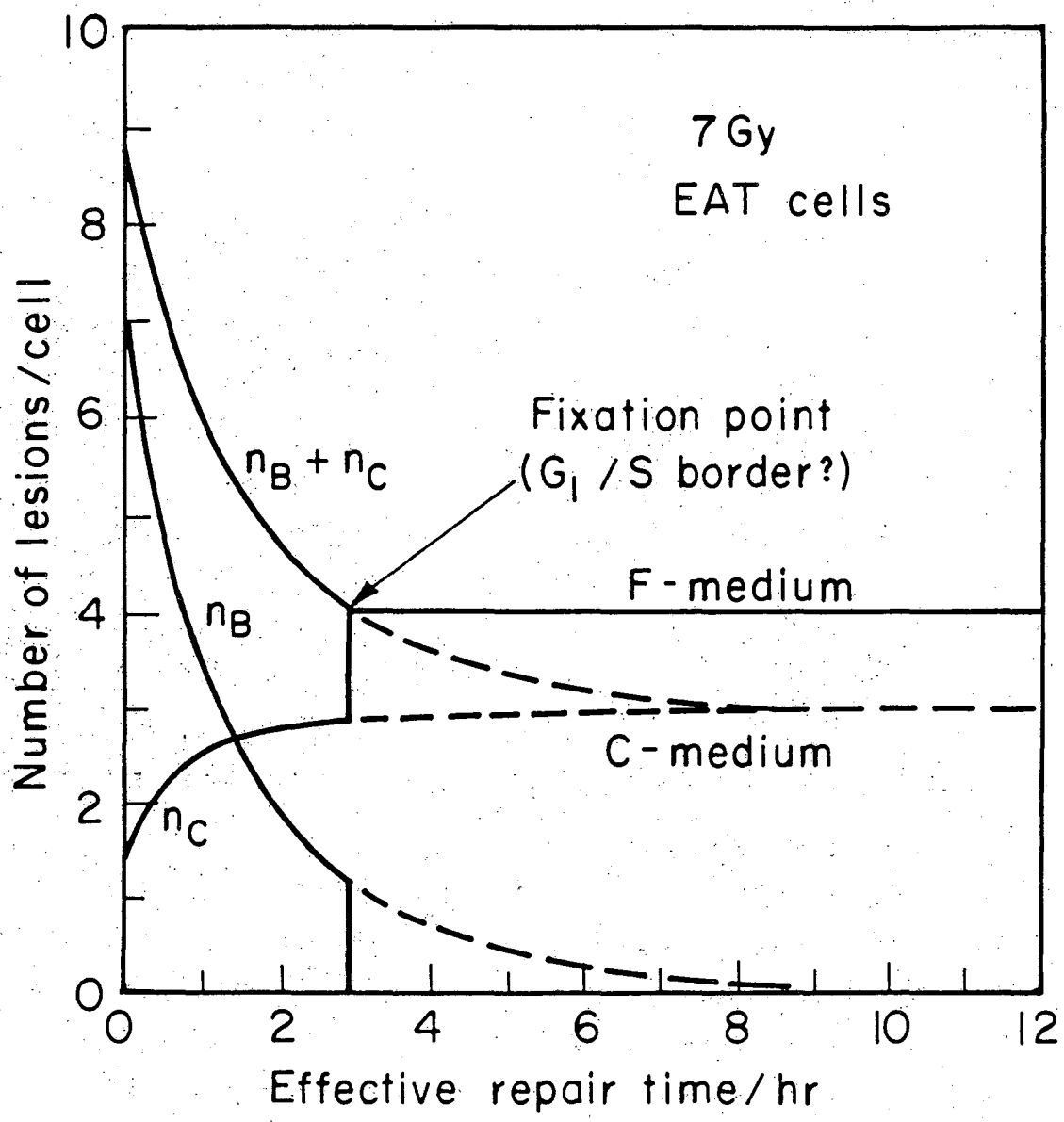

Figure 7. A comparison of the time course of the mean numbers of lethal (n_c) and potentially lethal (n_B) lesions in C, "conditioned," (dashed line) and F, fresh, or growth medium (solid line) after an absorbed dose of 7 Gy. A fixation point is assumed after 3 hours in fresh medium, i.e., all remaining potentially lethal elsions are fixed and become lethal at that point. (XBL 837-10638)

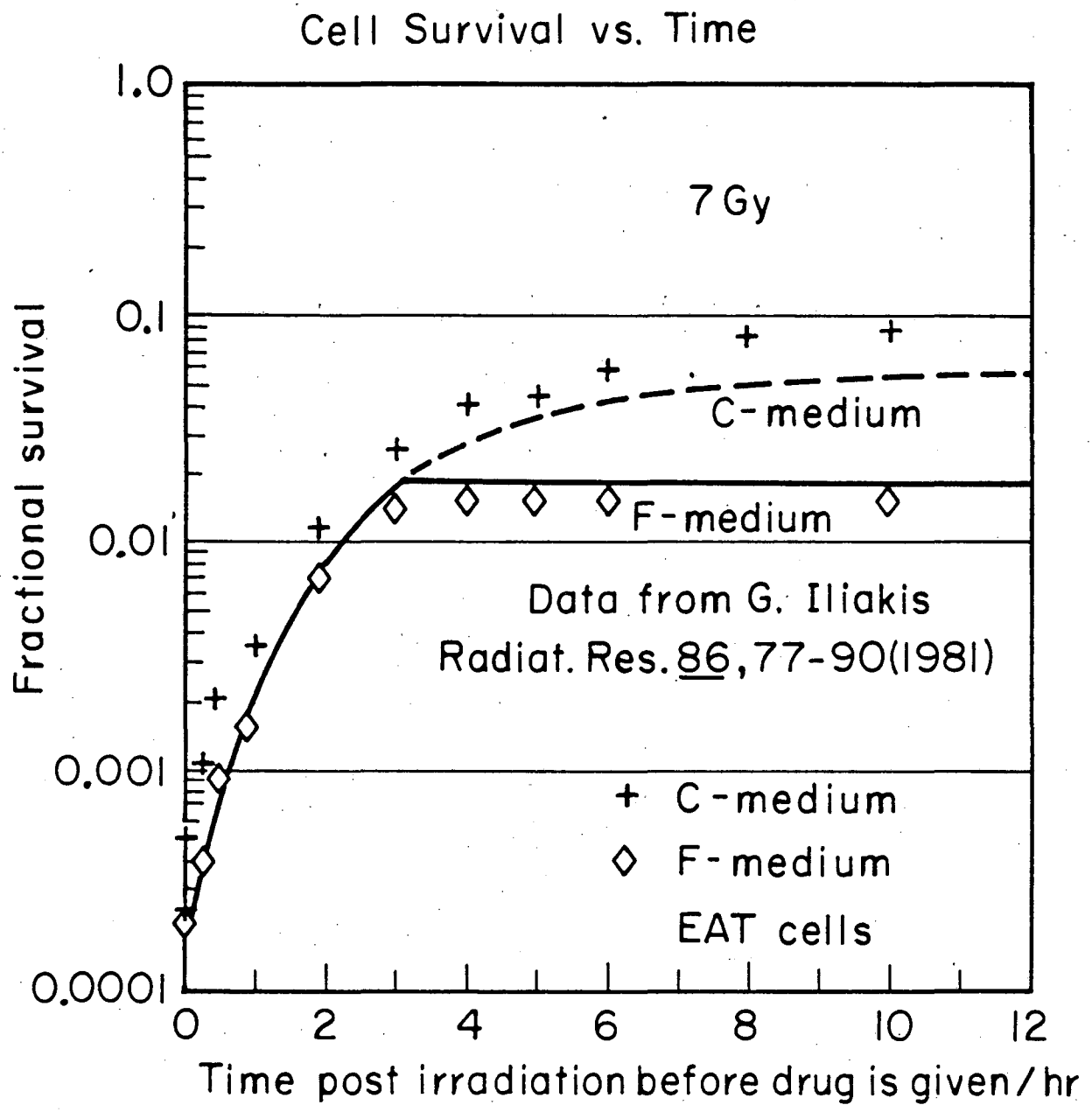

Figure 8. Cell survival as a function of time in "conditioned" (dashed curve) or fresh (solid curve) medium after an absorbed dose of 7 Gy. Comparison is made with experimental data from Ehrlich ascites tumor cells (Iliakis, 1981). (XBL 837-10639)

calculated survival compared with experimental data obtained by Iliakis using β-araA as the repair inhibiting drug (1981).

For a split dose experiment with interval Δt between doses, D_1 and D_2, we assume that the 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. If the cells are plated immediately, the survival equation becomes:

$$S(D_1,D_2,\Delta t) = \exp\left[-n_B(n_B(\eta_{AB}D_1,\Delta t) + \eta_{AB}D_2,t_o) - n_C(n_C(\eta_{AC}D_1,\Delta t) + \eta_{AC}D_2,t_o)\right] \quad (17)$$

Here, repair is occurring both within the repair interval, Δt, and after plating occurs, during a time t_o.

Figure 9 gives the time course of lesions in conditioned medium (top) and fresh medium (bottom) for a split dose experiment with a five-hour interval between doses. It is clear that after three hours in fresh medium, the time interval dose not affect the final survival. The difference in survival in the two media as a function of split dose interval is seen by comparing Figures 10 and 11. For the 2 Gy + 2 Gy split dose chosen, little difference is noted.

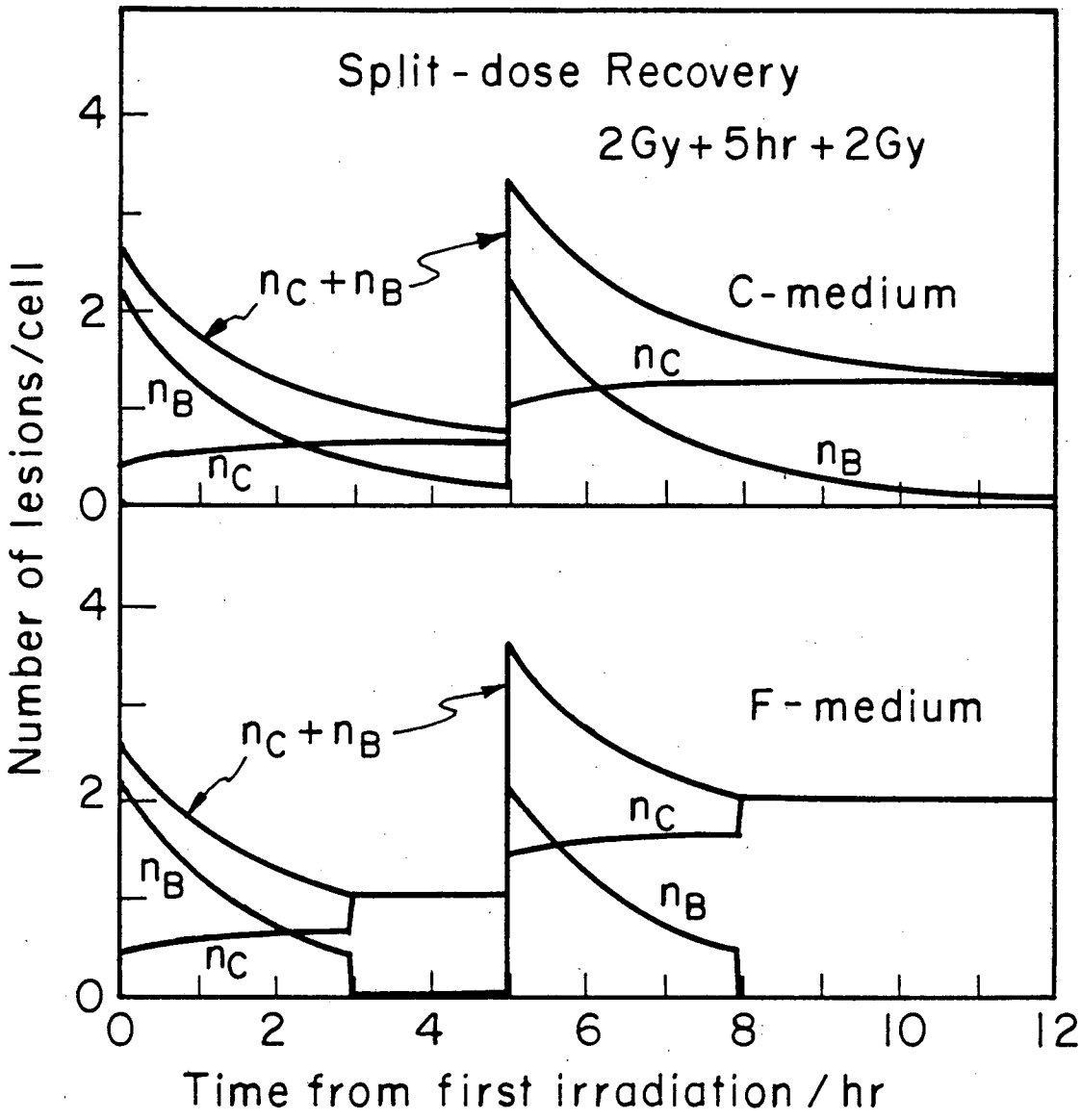

Figure 9. Time course of the mean number of lethal (n_C) and potentially lethal (n_B) lesions for a split dose recovery experiment in "conditioned" medium (top) and in fresh medium (bottom). The experiment assumes an absorbed dose of 2 Gy is followed by a repair period of 5 hours followed by another absorbed dose of 2 Gy. (XBL 836-3802)

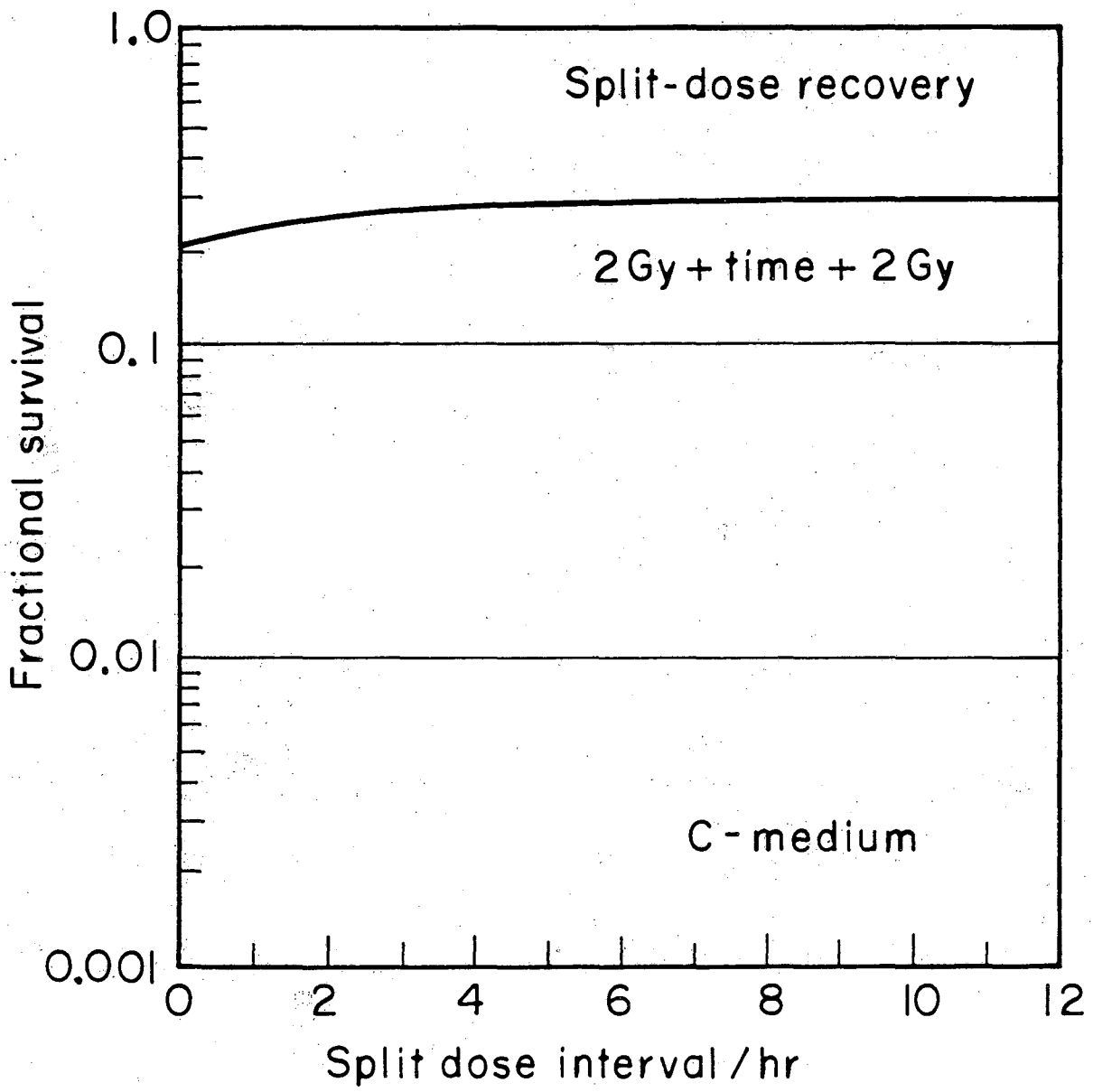

Figure 10. Cell survival as a function of repair interval for an absorbed dose of 2 Gy followed by 2 Gy in "conditioned" medium. (XBL 837-10640)

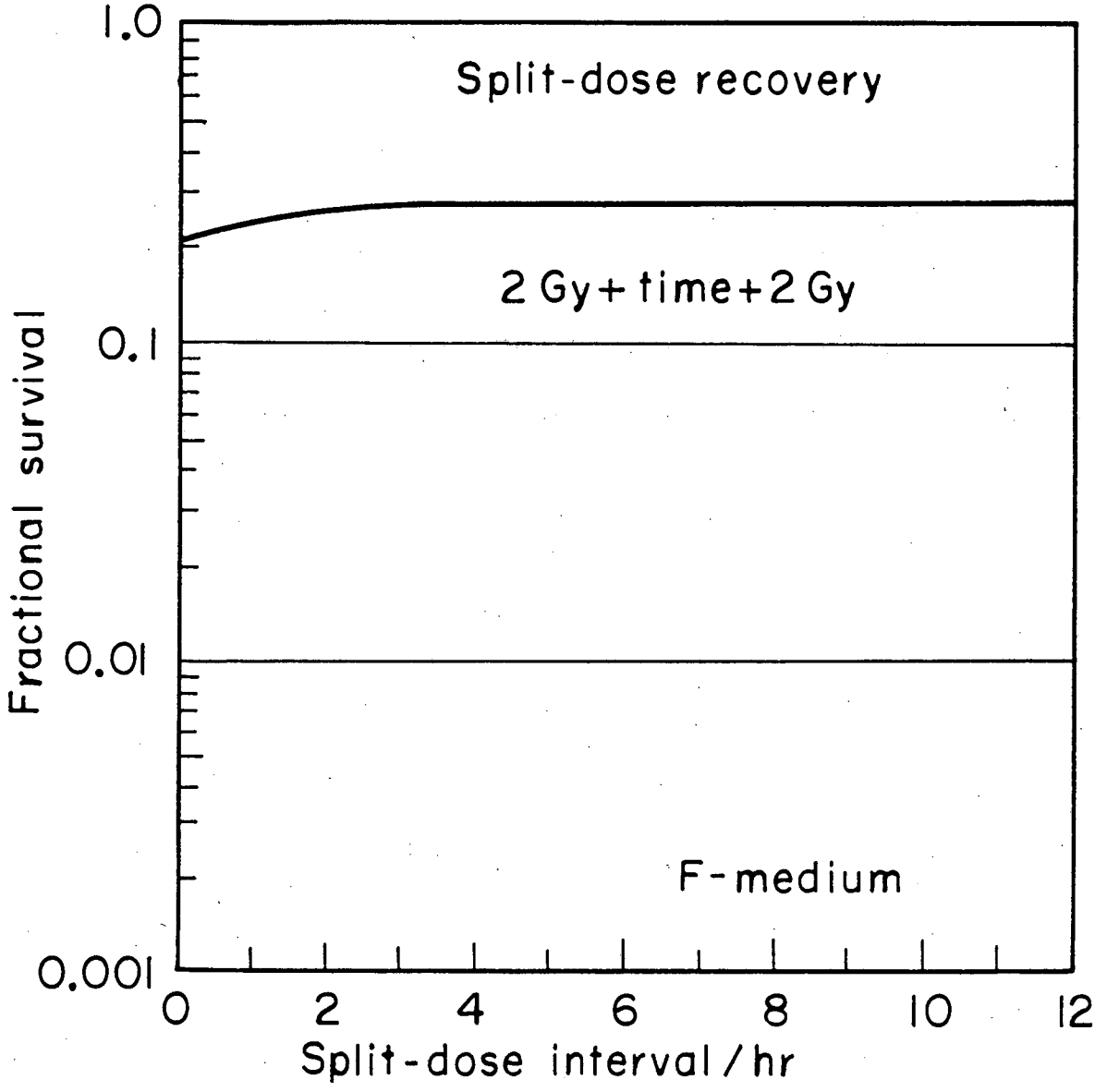

Figure 11. Cell survival as a function of repair interval for an absorbed dose of 2 Gy followed by 2 Gy followed by 2 Gy in fresh medium. (XBL 837-10641)

CONCLUSION

The lesions that are repaired (and misrepaired) in each type of experiment described above (delayed plating and split dose) are assumed to be the same. Thus, in this model the same (potentially lethal) lesions cause both sublethal and potentially lethal damage as defined in ICRU Report 30 (1979).[1] A crucial consideration in the expression of the damage is the kind of medium in which the cells are placed during the repair period. Fresh or growth medium (F-medium) is assumed to cause fixation of damage after about 3 hours, while no fixation (only misrepair) occurs in conditioned medium (C-medium).

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[1] Potentially lethal damage: damage, the lethal expression of which may be modified by alterations in postirradiation conditions; sublethal damage: cellular damage, the accumulation of which may lead to lethality.

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