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INTRODUCTION

There is general agreement that the effects of ionizing radiation on living cells are the results of discrete quantum mechanical interactions and transitions, and that some of the most important consequences of these effects are the eventual development of lesions in genetic material. Futhermore, we are acutely aware that many living organisms, but particularly eukaryotic cells, possess enzymatic repair mechanisms that can in the course of time heal or alter the lesions in genetic material and thus profoundly modify the eventual results of radiation exposure at the cellular level. It should logically follow that models of cellular radiobiological phenomena should consider the structure of genetic material and physical radiation interactions with it, the radiation chemical consequences of initial energy transfer, and the time structure of enzymatic interactions.

In spite of such realizations, many of the quantitative models for cell survival are concerned almost exclusively with the physics and statistics of initial energy deposition events, and attempt to correlate the eventual expression of biological effects directly with these events. Examples are the "target," "hit," (Lea 1955; Elkind and Sutton 1960; Widerøe 1966; Ehrenburg 1977) and the "dual action" theories (Jacobson 1957; Sinclair 1966; Neary 1965; Kellerer and Rossi 1972, 1978; Chadwick and Leenhouts 1973, 1978). A few models have incorporated time dependent parameters (Kellerer and Hug 1963; Dienes 1966; Payne and Garrett 1975; Pohlit 1975; Garrett and Payne 1978; Braby and Roesch 1978; and Niederer and Cunningham 1976).

In the repair-misrepair (RMR) model, we propose that the development of cellular biological effects from ionizing radiations has distinct and separable phases. For the present discussion, four phases are important.

1. The initial physical energy transfer and redistribution of energy by physical events.

2. Migration of the deposited energy and the establishment of long lived molecular lesions as a result of radiation chemistry.

3. Biochemical processes including repair or enhancement, coupled with progression of cells through various physiological states.

4. Genetic and evolutionary processes.

A time table of events of this kind was shown at this conference by Don Chapman, and our version can be seen in Figure 1. A consideration of the above time sequence of events reveals that the first two basic processes can be separated from the latter two.

Time Domains in Cell Inactivation by Ionizing Radiations

Radiation Physics

Briefly, the initial processes of radiation physics are comparable in their rate of occurrence to the time of passage by the ionizing particle or ray across an atom. Interactions begin at about 10^{-18} seconds . High energy primary electronic exchanges occur in fast sequence; the initial local energy deposition events are then gradually thermalized. There is a set of steps

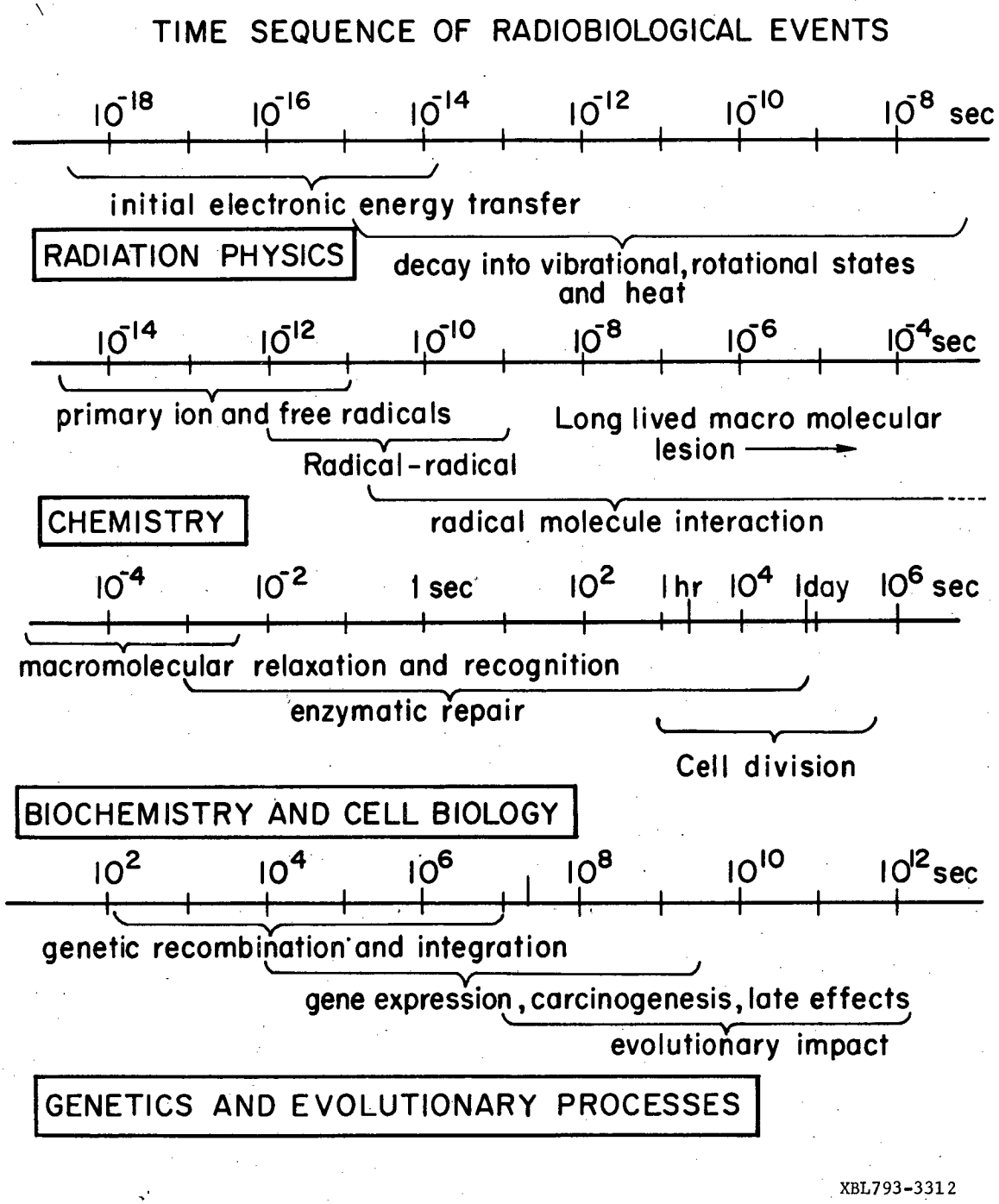


Figure 1

Time sequence of the radiobiological events found with cell irradiation, from the initial electronic energy transfer through late genetic effects. The physics events include time for heat transport.

involving reemission and reorganization of electronic energy levels. This is followed by energy transfer at the vibrational and rotational levels of molecules. The entire process including "thermalization" and heat flow is essentially complete in 10^{-8} seconds. An important role for radiation physics is then to quantitate the initial structure of energy deposition and the redistribution of energy in relation to molecular and cellular structures in living cells.

Radiation Chemistry

The radiation chemical phase overlaps the time sequence of radiation physics. This phase begins with the birth of highly reactive radicals and "short lived" free radicals of water and organic molecules. The free radicals then react both with each other and with the macromolecular structure of the genetic apparatus and other cell organelles. The radical reactions are diffusion controlled; in the course of the chemical events the initial physical characteristics of the tracks of ionizing particles are gradually lost. Chemical radiation modifiers and sensitizers act during this phase. We know from many experts in radiology, chemistry and biology that sensitizer action is important in the microsecond time domain. Recently Shenoy et al. (1975) have shown that the oxygen dependent damage in bacteria occurs in less than 100 microseconds. They failed to obtain an oxygen effect in mammalian cells when the oxygen was administered five milliseconds after the radiation exposure. We also have recent information that the modifying action of oxygen is limited by diffusion and that oxygen molecules can diffuse across one micrometer of cytoplasm in less than 10^{-3} seconds (Ling et al. 1978). From

a biological point of view, the goal of radiation chemistry is the prediction and measurement of the yield of specific "long lived" lesions in biologically important macromolecules such as DNA. Models for the radical chemistry phase were recently proposed by Magee (1979).

Radiation Biochemistry

Radiation biochemistry begins when long-lived macromolecular lesions have formed, for example in DNA. The essential steps are the recognition of lesions, mobilization of a sequence of specific enzymes to modify the lesions, energy dependent resynthesis of DNA, and the re-establishment of the appropriate tertiary structures. Among the known types of macromolecular lesions are single and double strand breaks in DNA, strand-to-strand cross-links, base alteration and dimerization, and protein DNA crosslinkages. The rate of repair and the enzymatic sequences for each type of lesion are different; the time scale for repair can range from minutes to days. The rate of repair of DNA lesions in human cells was recently discussed by Cleaver (1978).

Cell Biology and Genetics

Biochemical repair processes occur simultaneously with the progression of normal cellular physiology, which is usually delayed as a consequence of injury. The expression of certain radiation effects such as lethality or mutations depends on progression through the cell cycle. Repairing genetic damage and the appearance of "late" effects, though initially coupled with biochemical repair processes, may continue through several generations of cells.

Separation of Dose-Dependent and Time-Dependent Parameters

One of the difficulties with modeling radiobiological phenomena has been that they are usually described as functions of two independent variables: dose and time. A general treatment of dose and time dependent processes has resulted in mathematical complexities. However, we shall demonstrate that for the purpose of modeling cellular phenomena these two variables can be separated. We must first consider the manner in which cells recognize the lesions induced by deleterious agents and the limitations posed on radiobiological models by biological uncertainty.

Intracellular Recognition of Lesions

Our aim with the RMR model is to answer two questions: "when do cells "sense" that they are being damaged?" and "how do they respond to macromolecular injury?" The simple answers are that a certain amount of time must elapse after the lesions are made before the cell can recognize them as lesions. After recognition, the cells' enzymatic machinery and energetics are mobilized to repair or resynthesize the essential molecules involved.

In order to estimate the time needed to recognize the damage, assume that the damage consists of discrete lesions to the DNA. Several types of lesions have been demonstrated experimentally, but we wish to take strand breaks as examples. Elkind has estimated in his discussion at this conference that a mean lethal dose of x-rays produces about 10^3 single strand breaks and perhaps 50 double strand breaks in the DNA of a typical mammalian cell consisting of 10^9 base pairs. If this is the case, then on the average there

are about 10^6 pairs of unbroken phosphate bonds between neighboring single strand breaks and 2.4×10^7 pairs of unbroken bonds between double strand breaks. The number of repair enzyme molecules available can be estimated to be about 10^6 per mammalian cell.

We can now make one of two assumptions for the process of recognition: either the repair enzyme complex can recognize damage at a distance, or it is necessary for the enzyme complex to be at the site of injury before damage can be recognized. In this latter case we visualize that a typical repair enzyme complex moves up and down DNA continually testing its structure. We assume that the enzyme complex has a molecular weight of 10^5 daltons, that each enzyme complex has to test 10^3 base pairs, and that the process is diffusion limited. Calculations show that recognition of local damage may take on the average about one millisecond.

It is possible that the repair enzymes have a way of obtaining information about the occurrence of new lesions in DNA without first having to move to the actual site of the lesion. The enzyme (deployed adjacent to DNA, perhaps in nucleosomes) may sense the oscillations that are known to occur in DNA when it sustains local damage. For example, when a single strand break occurs, DNA removes the stress of supercoiling by uncoiling; when a double strand break occurs, DNA snaps open. The relaxation time of bacteriophage DNA is of the order of magnitude of 10^{-4} second (Pritchard and O'Konski 1977). The relaxation time in mammalian cellular DNA might be longer than in phage DNA since much more DNA is involved in a

more viscous milieu. We might assume that the oscillations caused by a strand break can be recognized in one relaxation time period, or 10^{-4} seconds. Figure 2 is a schematic drawing of this process.

Cells may be able to recognize various other types of lesions besides DNA lesions; membrane damage or significant concentrations of radiation products in the cytoplasm might also be recognized. However, reasonable calculations show that the timing for recognition and response to such events is probably not faster than the recognition of DNA nucleoprotein damage. The functional consequences of extranuclear damage are usually less serious for living cells than the consequences of a similar degree of nuclear damage.

Estimates for the rate of enzymatic repair can be made by considering the half times for repair in mammalian cells (7 to 15 min for single strand breaks and 80 to 90 min for double strand breaks) (Ritter et al. 1977; Roots et al. 1979) and the number of repair enzyme molecules available. Based on the discussion above and on Figure 1 we can make three conclusions which have contributed to the development of the RMR model.

1. The enzymatic apparatus of cells is likely to spend at least 10^{-4} seconds recognizing a radiation-induced lesion in its DNA after that lesion has been established. Biochemical responses to lesion are unlikely to be significant in less than 10^{-3} seconds, but by this time the great majority of initial electronic physical energy transfers and radiation chemical transformations are complete.

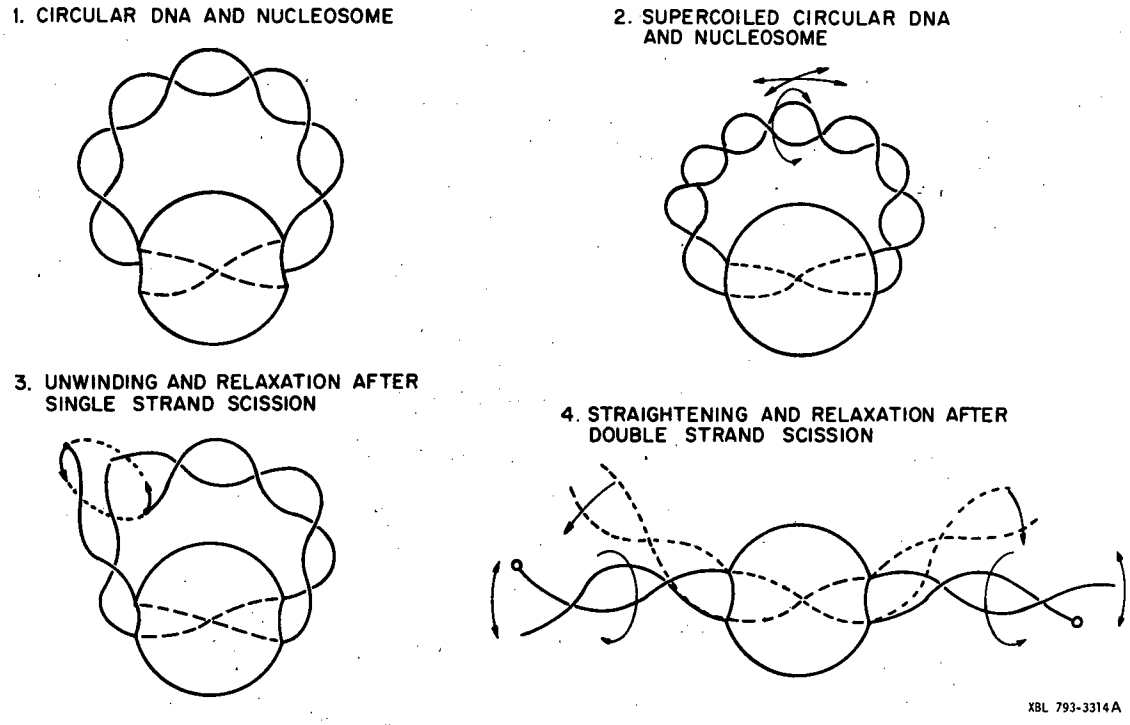


Figure 2

This figure shows the process of a DNA strand break. (1) Schematic view of DNA and nucleosome (based on nucleosomes in SV-40 virus). (2) The DNA is usually in supercoiled form. (3) The tension of supercoiling is released when a strand is broken. The unwinding from supercoiled form has characteristic relaxation times on the order of 10^{-4} seconds. (4) When a double-strand break is made, DNA unwinds and snaps open. The relaxation motions and change in coiling might be sensed by the production of stress in the macromolecular structure of nucleosomes.

2. The living cell can, however, recognize relatively long lived macromolecular lesions in its own structure, particularly on the genetic material, DNA. It is very likely that cells cannot recognize specific radiations, e.g., x-rays, neutrons, or heavy ions, as having distinctly separate properties because the specific interactions of these radiations occur too fast to be recognized by the cell. If two different deleterious physical or chemical agents produce the same kind of macromolecular lesions in DNA, it is likely the cell can not distinguish between these two agents.

3. We may treat quantitative models of the biological action of ionizing radiations in two distinct and separate phases: physicochemical and biochemical-genetic. One aim of physicochemical experiments (and of modeling) should be to ascertain the yield per cell of each specific type of macromolecular lesion as this yield depends on dose, initial absorbtion events, track structure, and eventual chemical modification. Given the yield of macromolecular lesions, the second, biochemical phase of modeling is to establish how these lesions relate to the eventual expression of biological effects.

Limits of Available Information as it Relates to the RMR Model: Biological Uncertainty

A salient feature of radiobiological phenonemena is that the effects are expressed with a considerable time delay after an initial physical energy transfer. In the intervening time we are seriously limited in our knowledge

about radiation induced lesions and their relationship to the fate of the cells. Various names have been used to denote lesions at the early stages, such as "sublethal lesions," "potentially lethal lesions," "sublesions," or "prelesions."

Analysis of DNA extracted from cells does reveal an average number of specific lesions by molecular weight measurements, and it can also demonstrate "repair" by rejoining with the same technique. However, techniques do not exist to assay the integrity of DNA and its base sequences in a single living cell. Because of the very small dimensions of individual codons, any physical technique that we could use for examining the DNA in a living cell would, by necessity, cause new lesions in the DNA. For this reason, it seems prudent to assume for modeling purposes that the fate of a given radiation induced macromolecular lesion is initially uncertain. Only after the cell attempts enzymatic repair will it be determined whether or not a given lesion will result in lethality or will be inconsequential. It seems logical that intracellular enzymatic structures of the cells have more information at the early stages of radiation injury than would the extracellular experimenter no matter what physical probe he uses.

Our model regards the fate of the early radiation induced lesions in cells as uncertain. We will introduce probability factors to describe whether these lesions can be perfectly repaired or if they lead to lethality due to imperfect misrepair, which includes incomplete repair.

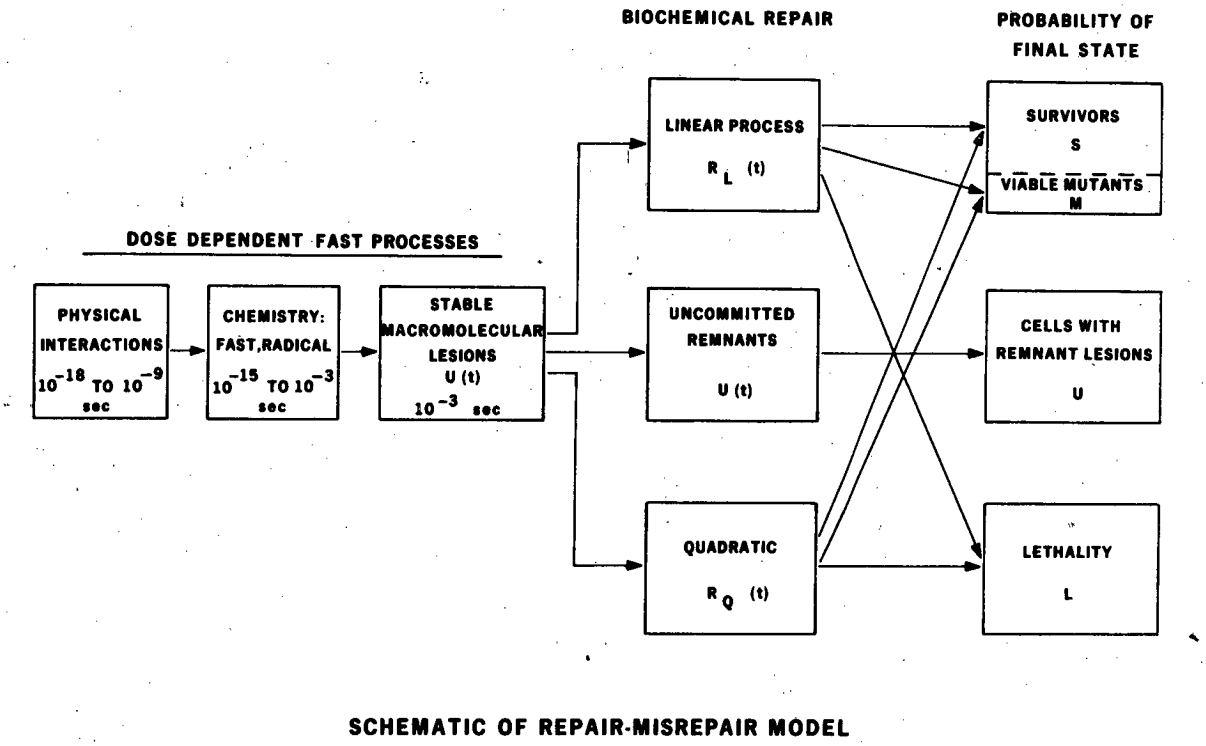
REPAIR MISREPAIR MODEL

The model describes the yield of relevant macromolecular lesions per cell as a function of dose (D); the time-dependent (t) transformations of these lesions; and the time- and dose-dependent probabilities for survival (S), lethality (L), and mutation (M).

U stands for "uncommitted," which is what the lesions are before they are subject to enzymatic repair and modification. Lesions with somewhat similar properties are described in other quantitative models (Garrett and Payne 1978; Pohlit 1975: Powers 1962: Laurie et al. 1972).

We know that various kinds of deleterious agents produce various classes of U lesions, each of which might potentially produce a variety of expressed biological effects. For clarity, this is restricted to the discussion of a single class of U lesions. A given class of U lesion can be identified with a specific molecular lesion if the time rate of its biological transformations, as predicted by the model, agrees with the average rate of change measured by molecular techniques. Additional types of U lesions can be introduced in the model when a single type of lesion cannot account for all experimental data.

The general scheme of the RMR model is shown in Figure 3. The physical and chemical interactions, shown on the left side of the figure, are interesting only because they determine the dose-dependent yield U_0. The model itself



SCHEMATIC OF REPAIR-MISREPAIR MODEL

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Figure 3

Schematic drawing of the repair-misrepair model, showing the dose-dependent fast processes, different types of biochemical repair, and the probabilities of the final states of the cell.

is concerned with time dependent transformations in the course of repair and with the probabilities that transformed states lead to the expression of biological effects.

We define R states (repair states) as the result of transformations of U states following enzymatic repair. R states are permanent in the sense that their presence may commit the cell to lethality, mutation, or survival. In the simplest form of the RMR model there are two R states: R_L is the yield of a linear repair process assumed to proceed as a monomolecular chemical reaction, and R_Q is the yield per cell of a repair process involving interaction between pairs of U lesions. R_Q is a "quadratic" repair process, and its rate is proportional to the square of the local density of U states. If the distribution of U lesions is uniform throughout the cell nucleus, then the rate of R_Q is proportional to $U(U-1) \simeq U^2$.

For a single, rapidly delivered dose of low linear energy transfer (LET) radiation the time dependent behavior of U is described by the first order quadratic differential equation:

$$\frac{dU}{dt} = -\lambda U(t) - kU^2(t) \qquad (1)$$

In this equation λ is the coefficient of linear repair and k is the coefficient of quadratic repair. Integrating between limits 0 and t we find:

$$U(0) - U(t) = \int_0^t \lambda U(t)dt + \int_0^t k\,U^2(t)dt \qquad (2)$$

With the definitions

$$R_L = \int_0^t \lambda U(t)dt$$

and

$$R_Q = \int_0^t k U^2(t)dt$$

we have

$$U(t) + R_L(t) + R_Q(t) = U(0) \tag{3}$$

Simple solutions exist for the decay of uncommitted U lesions and the growth of R states, with the assumption that for a specific cell type in a specific state λ and k are constant and independent of time and dose. Let $U(0) = U_0$; $R_L(0) = R_Q(0) = 0$; $U(\infty) = 0$; and $\varepsilon = \frac{\lambda}{k}$ the "repair ratio."

$$U = \frac{U_0 e^{-\lambda t}}{1 + \frac{U_0}{\varepsilon}(1 - e^{-\lambda t})} \tag{4}$$

$$R_L(t) = \varepsilon \ln \left[1 + \frac{U_0}{\varepsilon}(1 - e^{-\lambda t})\right] \tag{5}$$

$$R_Q(t) = \frac{U_0\left(1 + \frac{U_0}{\varepsilon}\right)(1 - e^{-\lambda t})}{1 + \frac{U_0}{\varepsilon}(1 - e^{-\lambda t})} - \varepsilon \ln\left[1 + \frac{U_0}{\varepsilon}(1 - e^{-\lambda t})\right] \quad (6)$$

Eurepair and Misrepair

A range of possible repair states occur after damage to cellular DNA. The cell may repair the damage accurately, making the DNA sequencing exactly like it was before radiation damage occurred. We call this type of true repair "eurepair." The other types of repair are variations of misrepair and range from viable mutants to alterations that eventually cause cell death. One form of misrepair is misreplication. The process of DNA synthesis and repair is often not completely accurate even in normal, unirradiated cells (Bernardi and Ninio 1978).

The states R_L and R_Q represent the products of biochemical repair. If U represents DNA strand breaks, for example, R_L and R_Q would be yields of reconstituted DNA with strand continuity unless for some reason the repair is unsuccessful or incomplete. Let ϕ represent the probability that linear repair is eurepair, and δ the probability that quadratic repair is eurepair. The probabilities of misrepair will be represented by coefficients $1 - \phi$ and $1 - \delta$. It is obvious that incomplete repair is also misrepair.

Table 1

Definitions for the Probabilities of Eurepair and Misrepair

	Eurepair		Misrepair	
	Symbol	Probability	Symbol	Probability
Linear repair process				
R_L	R_{LE}	ϕ	R_{LM}	$1-\phi$
Quadratic repair process	R_{QE}	δ	R_{QM}	$1-\delta$
R_Q				

Survival Probability S(t)

Equations (4) through (6) and the definitions found in Table 1 allow for the calculation of the probabilities of survival S(t) and lethality L(t). Assume that statistical variations in U, R_L, and R_Q are random. (Poisson statistics are used here; a more detailed discussion of statistical approaches is being prepared.) The probability of survival at time t clearly depends on the number of misrepaired lesions (R_{LM} and R_{QM}) and the number of unrepaired U lesions that a given cell can tolerate at the time of cell division. Survival is usually measured as colony formation (reproductive integrity) after several cell divisions to limit the time variable such as $t < t_{max}$ where t_{max} is the time interval allowed for repair.

The genetic constitution of cells may also be a factor for survival. In order to describe the survival of higher ploidy or of binucleated cells,

it may be necessary to consider additional constraints. Thus, survival probability should be considered separately for a variety of situations with specified constraints.

We have considered six different applications of the RMR model. These cases have been chosen to interpret a variety of types of radiobiological experiments. Each of these cases is briefly described below, and a detailed discussion for each will follow.

Case I: Assume that all linear repair is eurepair and that all quadratic repair results in lethal misrepair. This leads to the simplest RMR survival equation with two adjustable parameters: α , the yield of U lesions per rad, and ϵ , the repair ratio.

$$S = e^{-\alpha D}\left[1 + \frac{\alpha D}{\epsilon}\right]^{\epsilon} \qquad (7)$$

There are interesting similarities and differences between this survival expression and the multitarget single hit survival curves. Equation (7) is useful for fitting survival data from mammalian cells exposed to x-rays.

As an illustration of Case I, an analysis of the x-ray survival of various repairless mutants of yeast cells will be made.

Case II: Assume that fraction of linear repair is misrepair which causes more lethality than that found with Case I.

$$S = e^{-\alpha D}\left[1 + \frac{\alpha D}{\epsilon}\right]^{\epsilon\phi} \qquad (8)$$

In addition to α and ϵ there is a third adjustable parameter, ϕ, which was defined in Table I.

The survival probabilities of Case II are compared to the linear-quadratic survival equations. This form of survival equation is suitable for analysis of mammalian cell survival curves resulting from high LET radiations, and the analysis of survival as a function of cell age.

Case III: For the application of the RMR model to split dose and mixed modality exposures, we assume that a time interval τ separates two dose instalments D_1 and D_2. The survival equation is:

$$S(D_1, D_2, \tau, t) = L(D_1, \tau) \cdot S(D_2, t - \tau) \qquad (9)$$

At the end of period τ, remnant U lesions are added to the new lesions produced by dose D_2.

We will show an example of split dose experiments with x rays on mammalian cells in the following discussion. Mixed radiation exposures may be analyzed in a similar manner. The analysis of split-dose experiments uses the concept of remnant U lesions. The relationship of remnant lesions to the initial slope of the survival curves will be discussed.

Case IV: For the calculation of mutation probabilities, we shall assume that a specific mutation corresponds to a specific kind of misrepair. The frequency of mutation induction as a function of dose is then, in a simple case, proportional to the amount of misrepair that is occurring while the cell recovers from a dose of radiation.

Case V: Repair processes that depend on the magnitude of administered dose may also be considered with the RMR model. The same dose of radiation that causes U lesions might either inactivate or enhance repair processes. Hence we obtain survival curves for repair inactivation by allowing the coefficient ϵ to be a decaying function of dose.

A second example allows ϵ to increase as an increasing function of dose. This results in survival curve shapes that have been described from experiments with bacteria and algae as "SOS repair."

Case VI: We will discuss survival and lethality from ionizing radiations that are delivered in protracted fashion at constant dose rate. Repair is occurring while the dose is still being administered, and the cells are left with accumulated U lesions at the time when radiation is stopped. The RMR model predicts certain types of dose rate effects for protracted doses.

We will now proceed with a detailed discussion of each of the six cases of the RMR model.

Case I of the RMR Model

Case I is based on the idea that linear repair, e.g., the rejoining of two adjacent broken ends of DNA in order to reconstitute the original unbroken piece, is always eurepair. Quadratic repair, which is the rejoining of pieces of DNA which did not originally belong together before the cell was exposed to radiation, is always misrepair, in fact misrepair causing a lethal effect.

If all R_L is eurepair, $\phi = 1$. If all R_Q is lethal misrepair, $\delta = 0$. If remnant U lesions are lethal, then at time (t) any cell that has R_Q lesions is committed to die, and cells that have no R_Q or U lesions are committed to survive. According to Poisson statistics and based on equations (4) through (6).

$$S(t) = \exp\left(-R_Q - U\right) = e^{-U_0}\left[1 + \frac{U_0}{\varepsilon}\left(1 - e^{-\lambda t}\right)\right]^{\varepsilon} \tag{10}$$

With the designation of:

$$\gamma\left(U_0, t\right) = \frac{\left(1 + \frac{U_0}{\varepsilon}\right)\left(1 - e^{-\lambda t}\right)}{1 + \frac{U_0}{\varepsilon}\left(1 - e^{-\lambda t}\right)} \tag{11}$$

$$L(t) = 1 - \exp\left(-R_Q\right) = e^{-\gamma U_0}\left[1 + \frac{U_0}{\varepsilon}\left(1 - e^{-\lambda t}\right)\right]^{\varepsilon} \tag{12}$$

When $t \to \infty$ then $(S + L)_{t\to\infty} = 1$; which means that no U lesions are left, they have all been eurepaired or misrepaired. In Figure 4 the time dependence of the functions S(t) and L(t) are shown in the course of repair following a dose of radiation.

Dose Dependence of U and S

Dose does not explicitly enter into the survival probabilities (equations 10 through 12). However, U_0 is a function of dose. Generally, the dose dependent initial yield of U lesions might be approximated by a power series that is subject to experimental verification at the molecular level:

$$U_0 = \sum_{i=1}^{i\ max} \alpha_i D^i \qquad (13)$$

where α_i is a constant.

Without limiting the eventual applicability of the model, we shall restrict ourselves here to a discussion of single-strand DNA scissions and double-strand scissions produced by ionizing radiation. There is a variety of experiments available on the yield of strand breaks in microorganisms and in mammalian cells exposed to ionizing radiation. For example, in recent work on DNA of ϕX174 phage (Christensen et al. 1972; Hutchinson 1974; Corry and Cole 1973; Bonura et al. 1975; Sawada and Okada 1972; Veatch and Okada 1969) and on hamster cell DNA (Ritter et al. 1977), the yield of DNA strand breaks is proportional to dose. In phage, single-and double-strand breaks were measured separately, whereas in mammalian cells the sum of single- and double-strand breaks was

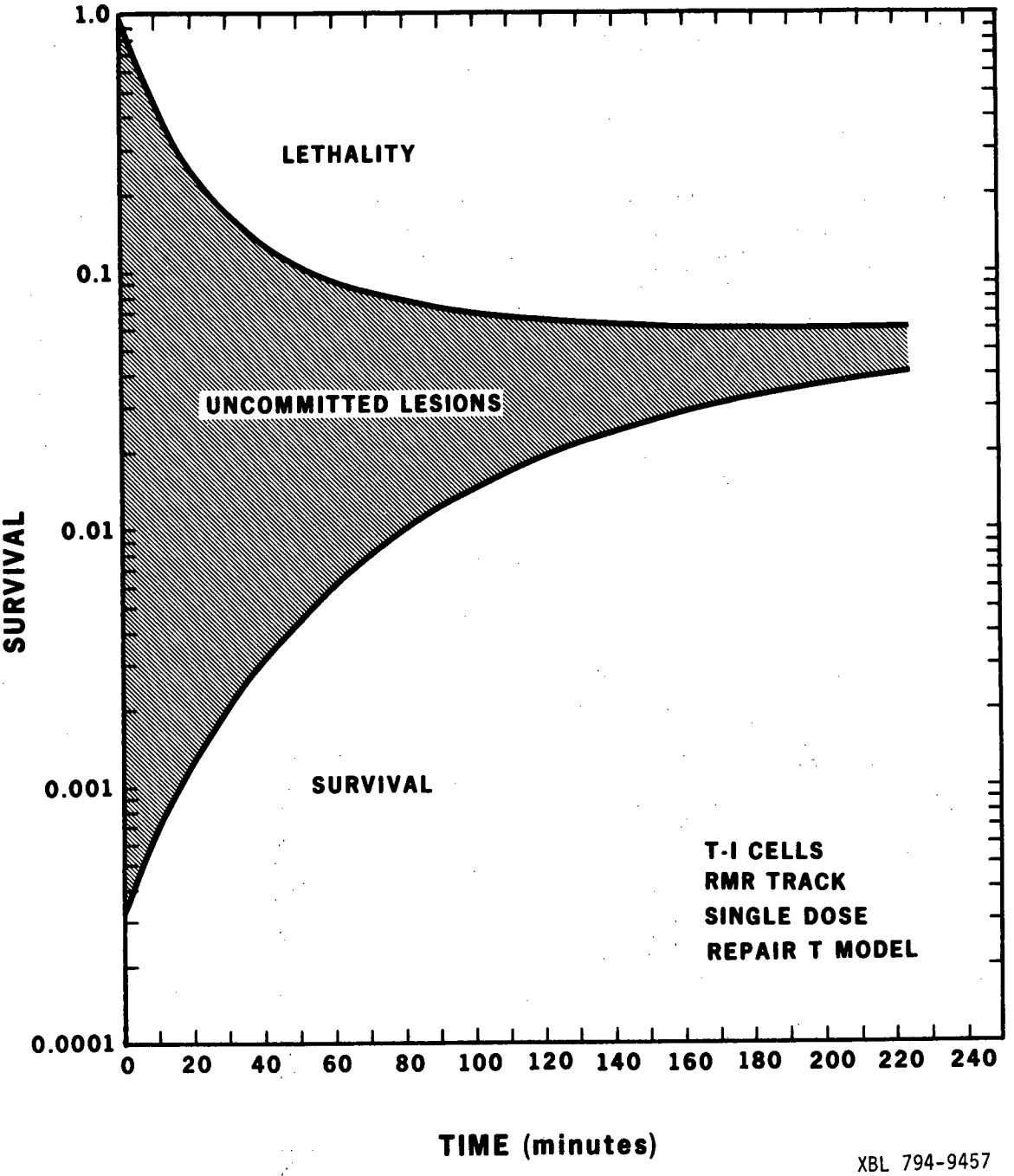

Figure 4

Time dependence of the probabilities for survival (S) and lethality (1 - L(t)) as a function of time elapsed after a single dose. The shape of the curves depends on the values λ and k. The number of cells with uncommitted lesions decreases with time.

measured. For both cases the yield of strand breaks was a linear function of dose:

$$U_0 = \alpha D \qquad (14)$$

where α is the yield of strand breaks per cell per unit dose. The proportionality holds regardless of the particle or radiation used, whether it is x-rays, carbon, or argon ion beams; α does vary with beam quality, however.

There are some observations which tend to favor a quadratic relationship for the yield of strand breaks:

$$U_0 = (\alpha_1 D) + (\alpha_2 D^2) \qquad (15)$$

where α_1, α_2 = constants

For example, Dugle et al. (1976) observed this a relationship for mammalian cell DNA with x rays at very high doses. Also certain models, e.g., the dual action theory (Kellerer and Rossi 1972; 1978) propose relationships similar to equation (15).

The RMR model can use any of the forms of dose dependence for U_0 (equations 13 through 15) and this model may in fact be helpful in deciding which equations express the yield of U_0 lesions most accurately. In the present paper, we use only the linear relationship of equation (14); most biochemical evidence supports this choice.

The Rates of Repair: λ and k

For DNA strand scission, the rate of repair has usually been found experimentally to be proportional to the number of strand breaks present in the cell, leading to simple time-dependent exponential relationships for the decay of strand breaks. For x-ray-induced strand breaks measured by the alkaline sucrose method, the half-life for repair at 37°C is 7 to 15 minutes. Ritter et al.(1977) found that after heavy-ion irradiation about 50% of the breaks repaired with an 80-minute half-life, and up to 20% of the breaks remained unrepaired in a 12-hour time span. The slower rate of repair after heavy ion exposures can be correlated with the increased incidence of double-strand scission. A variety of authors have demonstrated the repair of double-strand breaks (Hutchinson 1974; Corry and Cole 1973; Roots et al. 1979).

The term "double-strand scission" probably denotes a variety of lesions in mammalian cells which at some time or other following exposure to radiation reach a state where both strands of DNA are severed. Most methods to assay the number of DNA strand breaks are indirect. There is also an indication that some double-strand breaks remain unrepaired even after 12 hours or more incubation of the damaged cells. Roots et al. (1979) have correlated the fraction of unrepaired breaks to the relative biological effectiveness (RBE) of heavy ions for inhibition of reproductive integrity in human kidney cells. However, we do not have direct evidence at present whether the inability to repair causes death, or rather the cells that are dying have lost their ability to repair because of some other cause.

The increase in yield of DNA double strand scissions appears to be intimately connected to the increased biological effectiveness of alpha particles and of accelerated heavy ions. Although most of the double-strand lesions are repaired, this repair is measured at the chemical level as an increase in molecular weight of DNA fragments. No chemical information is available on whether or not the entire coded message is preserved during the double-strand repair process, i.e., whether the repair is eurepair or misrepair. If we draw a parallel between the production and repair of double-strand breaks and chromosome breakage and repair, it becomes obvious that at the chromosomal level certain types of rejoinings, e.g., deletions or translocations, relate intimately to the survival or death of the cells and to the possible presence of mutants. It seems straightforward to assume that DNA double-strand scissions may often rejoin with DNA deletions and DNA rejoinings between two abnormal sets of broken DNA strands. Neary (1965) has theorized that abnormal chromosome rejoinings are proportional to the square of the dose.

The form of the RMR model, as given in equation (1), is patterned to fit the above ideas. The linear repair constant λ could represent the rate at which the broken strands of the same DNA molecule rejoin, and should be mostly eurepair unless the repair process for some reason cannot be completed. The rate constant k could represent DNA deletions and exchanges. The values of λ and k cannot be evaluated in a single survival experiment; this can be done, however, in split-dose experiments.

Practical Survival Equation for Case I Derived from Equations (10), (14), and (15)

In Figure 4 we have plotted the time dependent probabilities for cell survival, equations 10 and 12, for a single exposure to dose D which caused $U_0 = \alpha D$ lesions. On this figure we see that as the cells become committed to survive, or die due to the formation of R_{LE} or R_{QM} states, the probability of finding cells with U lesions diminishes. The actual observations of survival probability usually are made at a much later time after the cells have gone through several divisions and the cells that survived have formed colonies.

We now introduce the factor T as a "time constraint" that depends on the maximum time available for repair, t_{max}:

$$T = 1 - e^{-\lambda t_{max}} \tag{16}$$

t_{max} might be set as the time available from exposure to the first mitosis. In this case $T < 1$; alternatively, t_{max} might be the time of some other event in the cell cycle where repair ceases.

$$S = e^{-\alpha D} \left[1 + \frac{\alpha DT}{\epsilon}\right]^{\epsilon} \tag{17}$$

Usually, if $\lambda t_{max} >> 1$, then $T \simeq 1$ and survival approximates equation (7):

$$S = e^{-\alpha D} \left[1 + \frac{\alpha D}{\epsilon}\right]^{\epsilon}$$

We find that this survival equation fits experimental data on lethal effects of low-LET radiation exceedingly well. For example, a survival curve for V-79 hamster cells exposed to x rays, obtained in our laboratory by Yang is shown in Figure 5 together with an RMR survival curve fitted by nonlinear least squares method. In comparison, the linear multitarget (LMT) model (Elkind and Sutton 1960) and the repair saturation model (Green and Burki 1972) fit less well because of lack of fit in the "shoulder" region; the linear quadratic model deviates from the experimental data either in the shoulder region or at high doses.

Figure 6 demonstrates the manner in which the survival curves drawn from equation (10) or (7) vary when the repair parameters change. A hypothetical mammalian cell similar to v-79 hamster cells was modeled. The yield constant α remained the same for all the graphs. In Figure 6A, which uses equation (7), the repair constant ϵ was varied. When $\epsilon = 0$, an exponential survival was obtained; as ϵ is increased the survival curves have increasing shoulders and decreasing slopes at high dose levels.

Figure 6B allows variation of the time t_{max} of equation (10) while keeping α and ϵ constant. If the repair time is zero, the survival curve is exponential as in the case of $\epsilon = 0$. With increasing t_{max}, different survival curves are obtained with different initial slopes approximating the case of $t_{max} \to \infty$. This case corresponds to $T = 1$ which has zero initial slope.

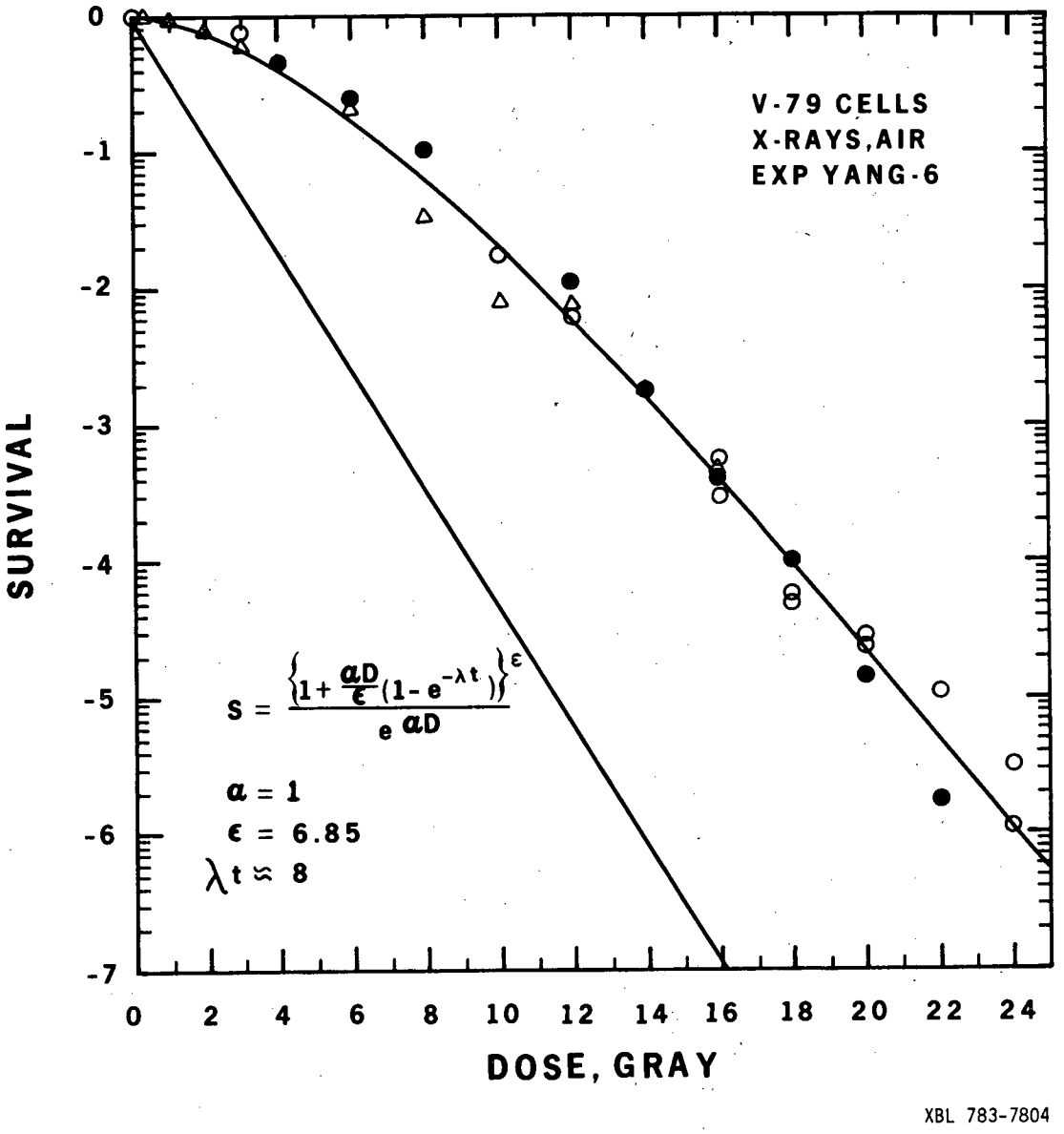

Figure 5

Survival of V-79 cells exposed to x rays (taken from the work of T. Yang). The solid line throught the experimental points is a fit by the RMR model. The exponential curve corresponds to $e^{-\alpha D}$.

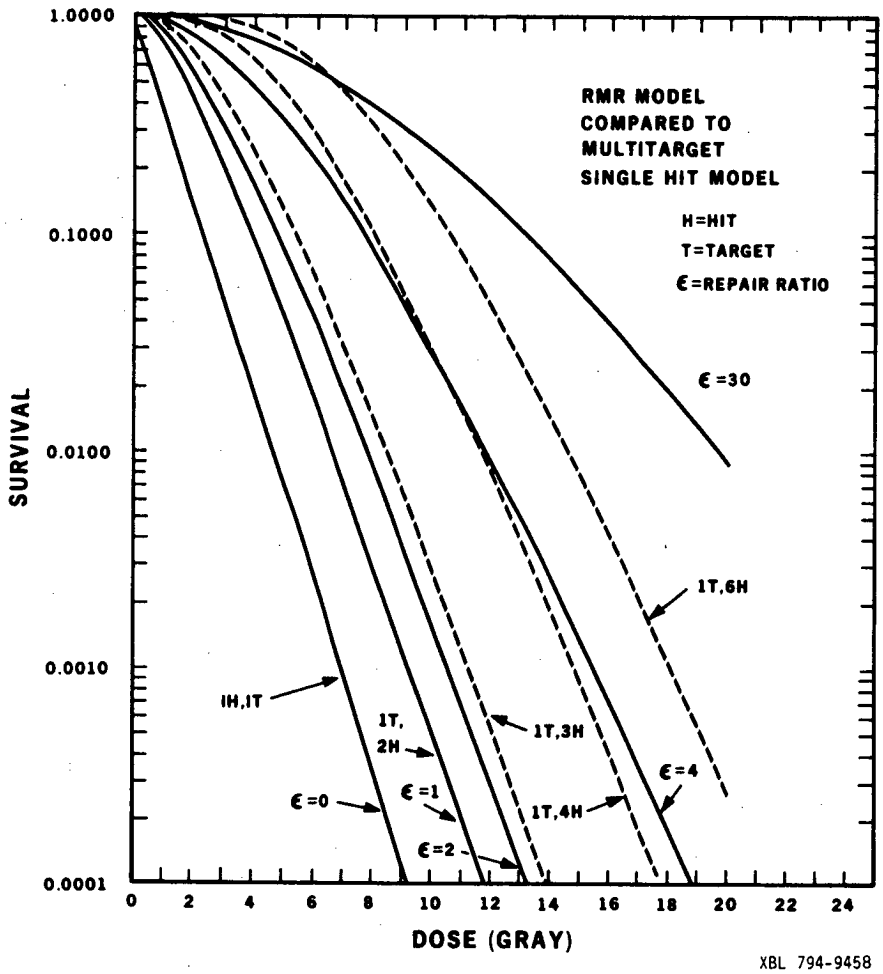

Figure 6A

Theoretical RMR survival curves according to the equation:

$$S = e^{-\alpha D}\left[1 + \frac{\alpha D}{\varepsilon}\right]^{\varepsilon}$$

are represented by solid line. The value α was kept constant, and ε, the repair ratio, was varied from 0 to 30. When $\varepsilon = 0$, there is no repair; when ε is large then linear eurepair is much more important than quadratic misrepair. The dotted lines represent the well-known single target, multihit survival equation:

$$S = e^{-\alpha D}\left[1 + D + \frac{(\alpha D)^2}{2!} + \ldots + \frac{(\alpha D)^2}{i!}\right]$$

The one-target single-hit curve is identical with the RMR survival curve when $\varepsilon = 0$. The one-target two-hit curve is identical with the RMR curve when $\varepsilon = 1$. At higher hit numbers there are significant differences between the two models both at low and at high dose levels.

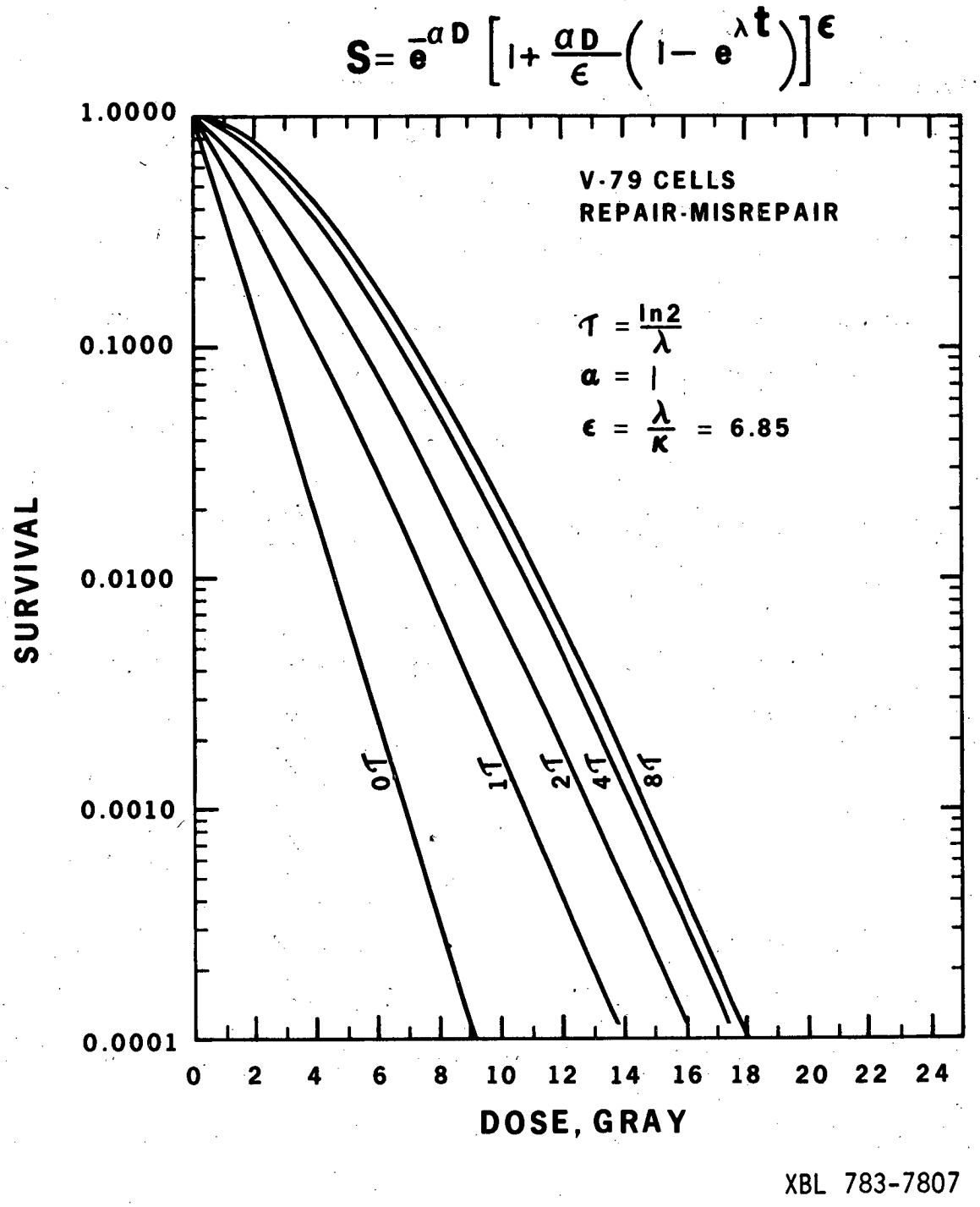

Figure 6B

The manner in which cells become committed to survive as repair proceeds in time. The coefficients α and ϵ were held constant, and the time available for repair was varied.

Comparison with Conventional Single Target Multihit Theory

Conventional target theory with m number of hits and α inactivation constant gives the following survival equation:

$$S_{(\text{target})}(m) = e^{-\alpha D} \sum_{i=0}^{m-1} \frac{(\alpha D)^i}{i!} \tag{18}$$

We can compare this directly with Case I if we expand equation (7) in the form of a power series. For the comparison, assuming $\epsilon = m - 1$ we obtain:

$$S_{RMR}(\epsilon = m - 1) = e^{-\alpha D} \sum_{i=o}^{m-1} \frac{(m - 1)!}{i!\,(m - 1 - i)!} \left(\frac{\alpha D}{m-1}\right)^i \tag{19}$$

Expressions (18) and (19) are similar except that the terms of (19) are smaller by the factor:

$$\frac{(m - 1)!}{(m - 1)^i\,(m - 1 - i)!}$$

When we deal with a "single hit" survival curve $S = e^{-\alpha D}$, both expressions are the same. In the RMR model, we claim that $\epsilon = 0$; there is no repair. Both models agree that either a single U lesion or a single hit kills the cell.

A two hit survival curve (m = 2) gives the same analytical form as $\epsilon = 1$. In this case, the multihit equation would claim that the cell could

always tolerate one relevant lesion, never two or more. The RMR model states that $\epsilon = 1 = \frac{\lambda}{k}$, therefore the coefficients for linear repair and quadratic misrepair are equal.

The dashed lines on Figure 6A are single target multiple-hit survival curves for the cases discussed above. Note that neither the constants D_q or D_0 of the target theory have a meaning for the RMR model; there is no "final" slope to measure D_0 because the survival curves are continually bending. For the same reason, it is not valid to extrapolate the survival curve to zero dose in order to obtain the extrapolation number m.

Survival Curves of Cells with Genetic Defects in the Repair Mechanism

As an example of the use of the RMR method and to illustrate the validity of some of the concepts used, we have used the data of Ho and Mortimer (1973) on the x-ray survival curves of genetically tetraploid Saccharomyces cerevisiae (Ho 1976). These authors demonstrated that a mechanism for the lethal effect in these cells was the production of double strand breaks in the nuclear DNA, but that in the wild type (+) of DNA, efficient repair mechanisms existed to repair double-strand breaks. Several repair deficient mutants were isolated; one of these (rad 52) was incorporated in the genome of five different yeast strains. Rad 52 is a repair-deficient gene, whereas the wild type gene can repair. The gene dose of rad 52 was 0, 1, 2, 3 or 4 in the

five different mutants; whereas the wild type gene went from 4(+) to 0(+)*.

Figure 7 shows survival curves of the type of equation (7) fitted for each strain by the least squares methods. The strain with four rad 52 genes had an almost pure exponential survival curve, showing very little repair. In the other strains , the yield parameter (α) was constant, whereas the repair parameter (ϵ) increased rapidly with gene dose. A plausible interpretation of these data is the assumption that the availability of the enzymes responsible for linear eurepair increased with dose of the wild type (+) gene, while intrinsic sensitivity of the genome for U lesions (α) and the rate of quadratic misrepair remained approximately constant. These experiments yielded only the value for $\epsilon = \frac{\lambda}{k}$; more could be learned about the values of λ and k in split dose experiments (see Case III of the RMR model, which follows).

Case II of the RMR Model

It is necessary to extend the treatment of the RMR model to situations where linear repair is <u>not</u> always eurepair. For example, even though a repair enzyme may attach to a U_0 lesion in a normal manner, it may be unable to complete repair. This would count as misrepair.

*Footnote: The genetic designation of the tetraploid strains of <u>S. cerevisiae</u> used was:

X3423	(X3406-1D x X3406-1A)	a/ a/ α/ α/ +/ +/ +/ +/
XK11	(X3406-1D x X3443-6A)	a/ a/ α/ α/ +/ +/ +/rad 52/
XK12	(X3406-1D x X3443-2B)	a/ a/ α/ α/ +/ +/rad 52/rad 52/
XK13	(X3443-11B x X3443-6A)	a/ a/ α/ α/ +/ rad 52/rad 52/rad 52/
X3452	(X3443-11B x X3443-2B)	a/ a/ α/ α/ rad 52/rad 52/rad 52/rad 52/

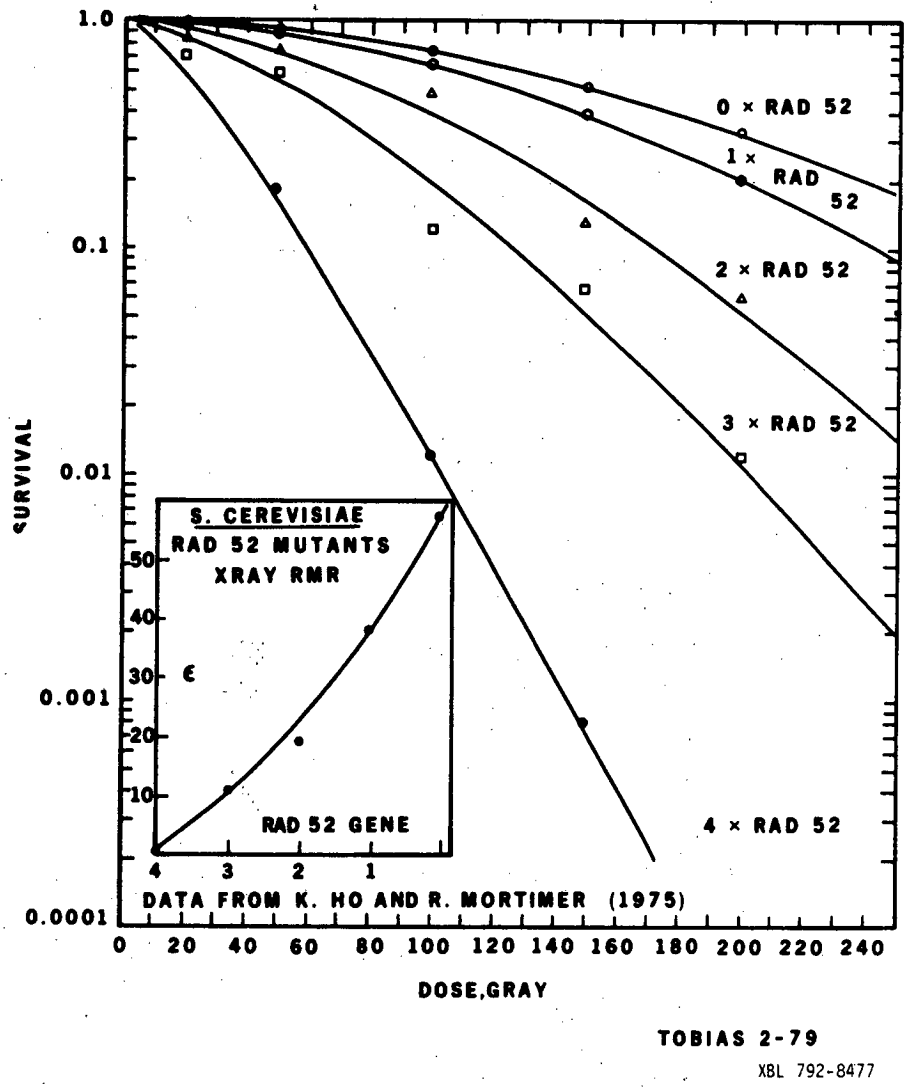

Figure 7

Experimental survival curves for tetraploid yeast cells (from the work of Ho and Mortimer 1973), fitted by the RMR model. Rad 52 is a repairless gene. The survival curves vary with gene dose in a manner generally in agreement with the RMR model. With α and k fixed, the values for ε are given in the insert. If we assume that ε measures the repair rate and that ε is proportional to the repair enzymes, then it appears that the amount of repair enzyme available increases approximately proportionally to the gene dose of the wild type gene (+).

The analysis that follows is an obvious simplification of the very complex repair mechanisms that are known to occur in nature. Let $\phi \leq 1$ be the probability that linear repair (R_L) is eurepair (see Table 1).

We have shown elsewhere (Tobias 1978) that in Case II, the RMR survival probability given in equation (7) can be modified to equation (8):

$$S = e^{-\alpha D} \left[1 + \frac{\alpha D}{\epsilon}\right]^{\epsilon\phi}$$

An important consequence of equation (8) is that at low doses the survival curves have a finite negative slope;

$$\left(\frac{dS}{dD}\right)_0 = -\alpha(1 - \phi) \tag{20}$$

In Figure 8A theoretical survival curves are plotted according to equation (8); the value ϵ is kept constant. When ϕ is zero, the survival is exponential. When $\phi = 1$, the curve is the same as that described by equation (7) and has zero initial slope. The family of curves with intermediate values of ϕ all had negative initial slopes; the slope gradually decreases as ϕ is increased.

We compared Case II to the linear quadratic survival equations (LQ) of Chadwick and Leenhouts (1973; 1978). Let x and y represent constant coefficients in the linear quadratic survival model expressed by the survival probability S_{LQ}:

$$S_{LQ} = e^{-xD-yD^2} \tag{21}$$

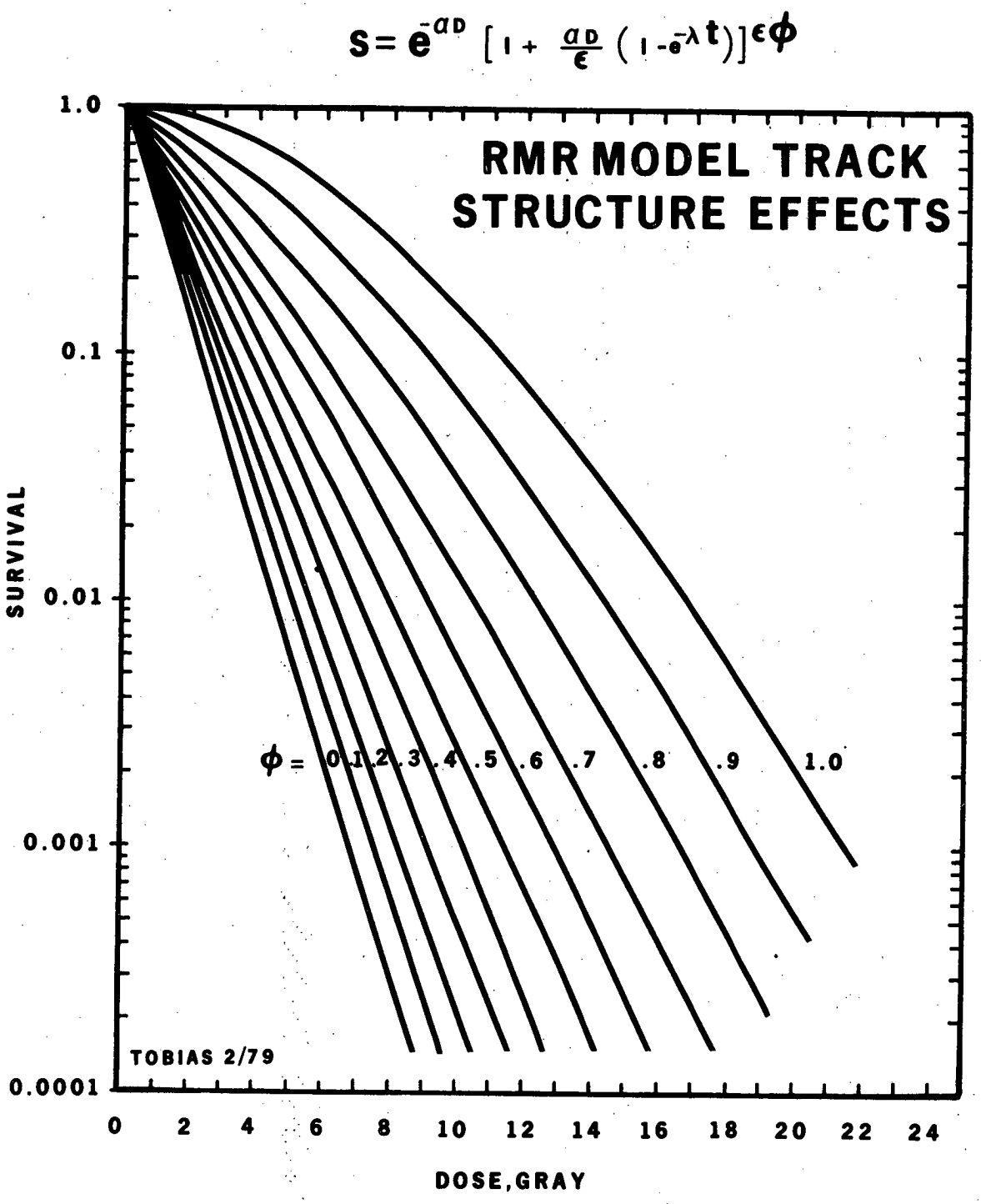

Figure 8A

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Theoretical survival curves and their dependence on the constant ϕ, which signifies the portion of linear eurepair. A single heavy ion produces several lesions along its track. The coefficient ϕ decreases as LET increases, and it measures the probability that all lesions produced in a single track are eurepaired.

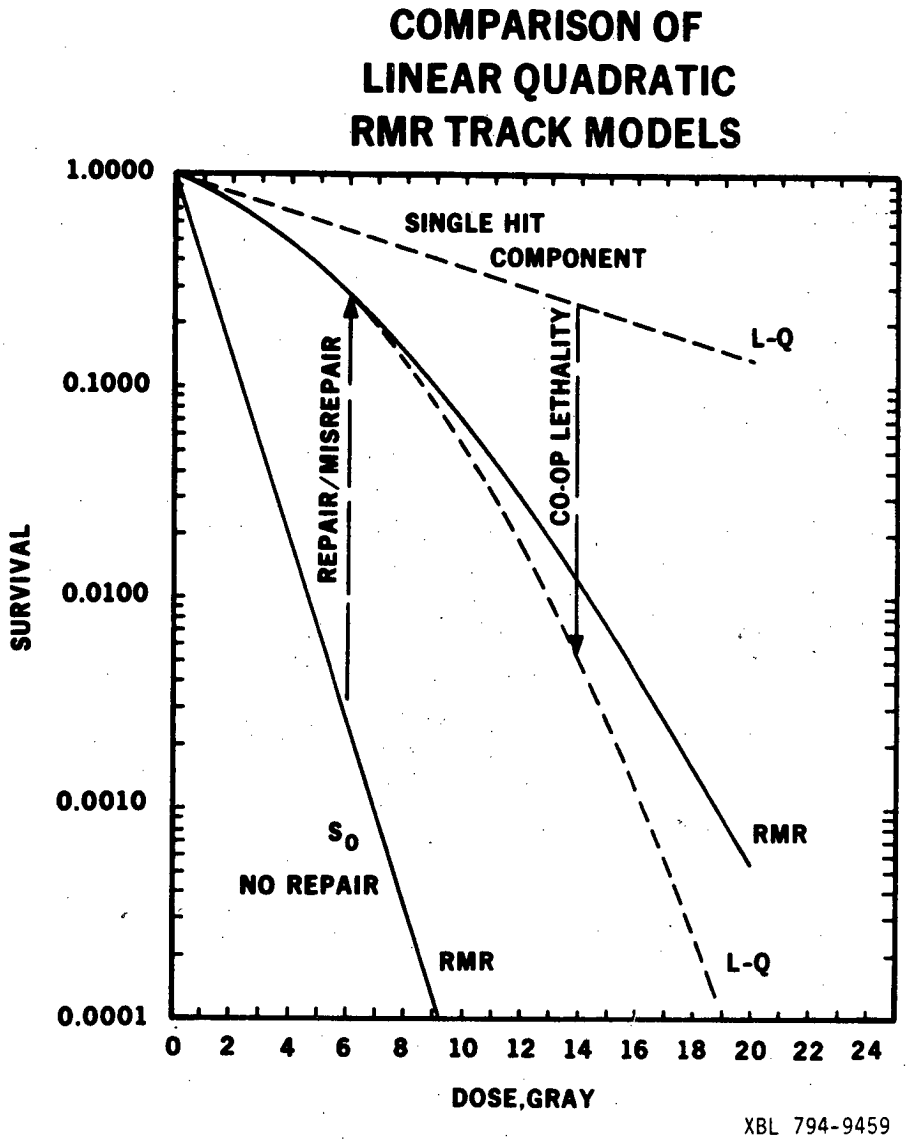

Figure 8B

Comparison of survival according to the RMR and linear-quadratic models. Continuous lines represent RMR curves, S_0 without repair and RMR with repair. The RMR curve is above the S_0 curve; repair helps survival. The linear-quadratic survival (equation 21) is indicated by dashed lines. The straight line, which corresponds to the initial slope, is usually interpreted as being due to single-hit irreparable or irreversible lesions. The lower dashed curve indicates the cooperative lethality due to the quadratic term. If the initial slopes are the same, then at very large doses the survival due to the linear-quadratic model always dips below survival due to the RMR model.

In Figure 8B we compared survival probabilities of equations (8) and (21), adjusted in such a manner that the initial slope of the survival curves is the same.

Equating the initial slopes gives:

$$x = \alpha(1 - \phi) \tag{22}$$

The usual interpretation of x according to the LQ model is that it represents the yield of irreparable lesions per unit dose. The RMR model initially has no irreparable lesions, but in the course of time a fraction $(1-\phi)$ of the initial U_0 lesions D are misrepaired. The RMR model has a repair term:

$$\left[1 + \frac{\alpha D}{\epsilon}\right]^{\epsilon\phi} \geq 1 \tag{23}$$

which is greater than one if ϕ is positive, signifying repair of lesions.

On the other hand the factor $e^{-\gamma D^2}$ of the LQ model may be regarded as a "potentiation term." Comparison of the two models in Figure 8B indicates that the survival due to the LQ model becomes progressively lower than the RMR survival at high doses. The slope of the RMR survival curve at large doses is always less steep than the slope of the RMR survival curve without the repair term, equation (23).

Equation (8) can also be used to describe enhancement of damage by changing the algebraic sign of ϕ from positive to negative. Enhancement corres-

ponds to an increase in the number of U lesions over what has been initially produced by a dose of radiation. Enhancement may occur as a result of enzymatic action. RMR "enhancement" survival curves would lie well below the exponential curve S_0 of Figure 8B.

The Interpretation of Survival Data Obtained with Heavy Ions

We have analyzed survival data obtained from human kidney cells irradiated with a variety of accelerated heavy ions at the Bevalac accelerator (Blakely et al. 1979).

Control x-ray data were fitted to the RMR model equation (8); the value of ϕ was nearly 1. Data from high LET radiations were then analyzed by nonlinear least square fitting of two constants: α and ϕ. We assumed that ϵ is the same regardless of particle velocity and LET, so that values obtained at low LET for ϵ were used to analyze the high-LET data.

We have used a neon beam of 425 MeV/amu nominal kinetic energy per nucleon. This beam has a useful range penetration of about 15 g/cm^2 in water. Survival curves were obtained along the Bragg ionization curve at eight different residual range values from 0.1 g/cm^2 to 12 g/cm^2 measured relative to the Bragg peak LET values ranged between 30 and 400 keV/μm. Figure 9 shows RMR fits to each of the survival curves. Note that the curves had less and less shoulder as the residual range was decreased and the LET increased.

In Figure 10 the RMR coefficients are analyzed as a function of the mean LET_∞. The beam was contaminated at low residual range values by primary

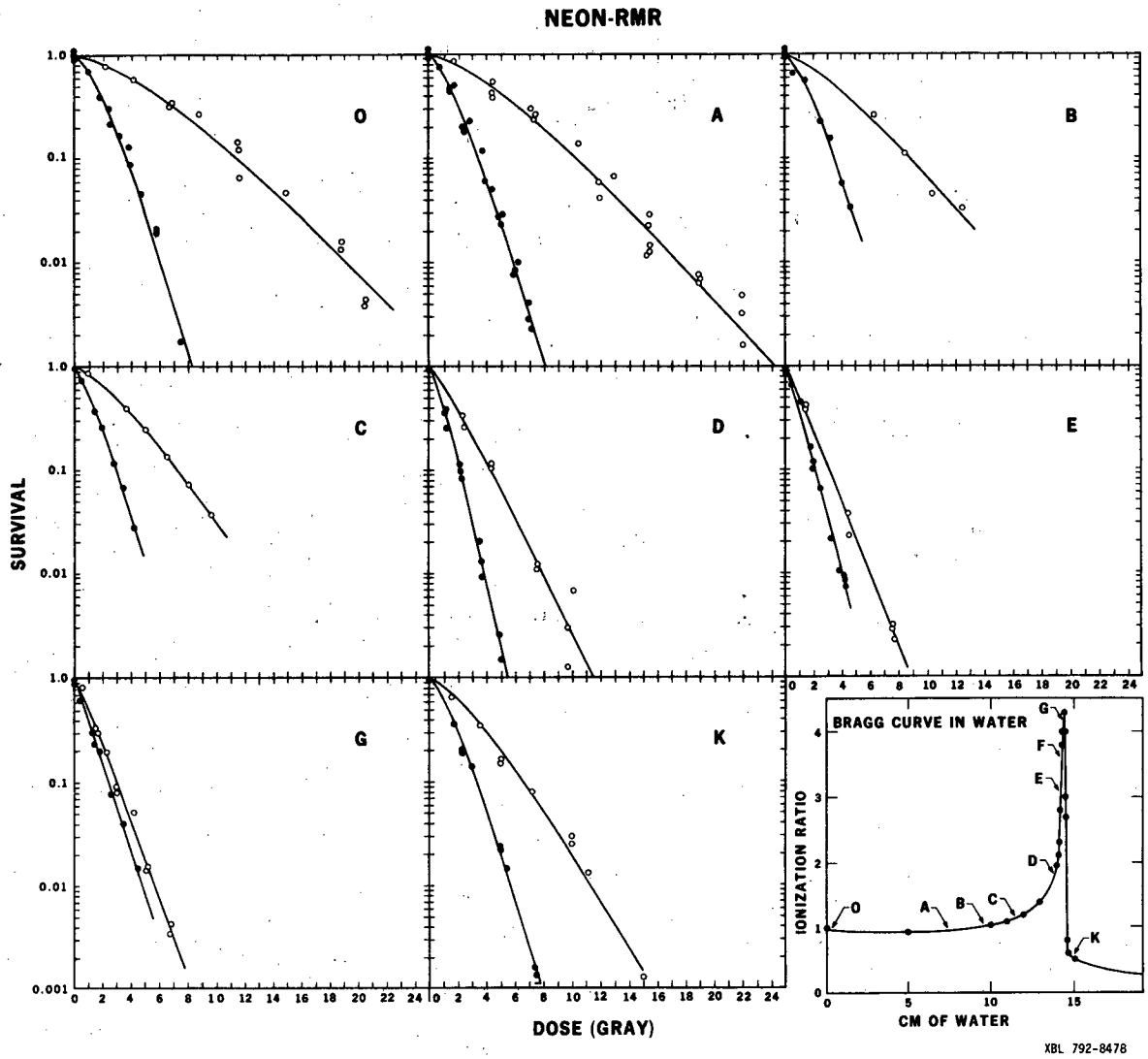

Figure 9

Experimental survival curves for T-1 kidney cells in air and in nitrogen. The cells have been exposed to neon beams of various residual-range values. The Bragg ionization curve at the bottom right panel shows the residual ranges, O through K, at which exposures were made. Solid squares indicate exposures in air; open squares indicate exposures in anoxic conditions. Note that the cells are more sensitive to neon particles near the Bragg peak and the oxygen effect is also reduced. The solid lines through the points are RMR least-squares fits.

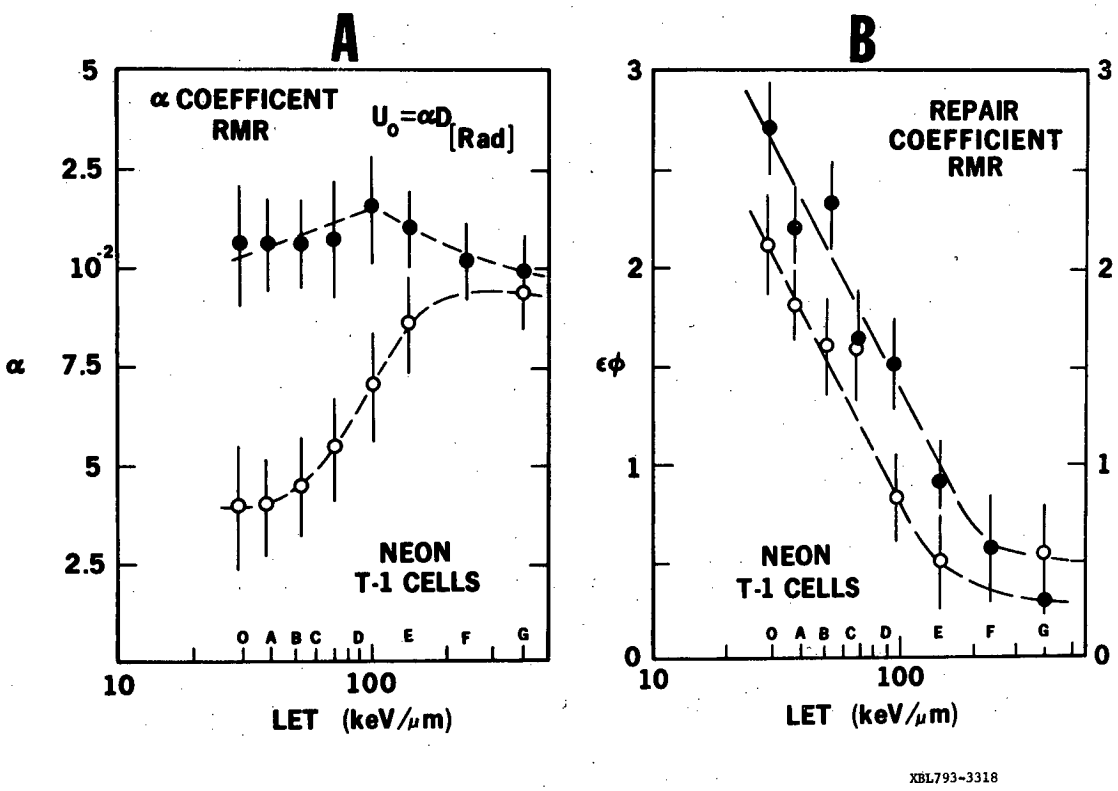

Figure 10A

Values of α for the experiments in Figure 9: air, ●; nitrogen, o. Since α is an indicator of U_0 lesions, we conclude that this yield increases with LET under anaerobic conditions. In air, however, the yield of U_0 lesions per unit dose is almost independent of LET.

Figure 10B

Decrease in the exponent $\phi\varepsilon$ of the survival equation as a function of LET: air, ●; nitrogen, o. The decrease of $\phi\varepsilon$ indicates that there is much less eurepair at high LET than at low LET.

beam fragmentation. In Figure 10A the coefficient α , which measures the yield U_0 lesions per cell, is plotted for exposures performed in air and those under hypoxic conditions.

If we consider the hypoxic conditions first, we see that α increases rapidly until it levels off above 100 keV/μm. In the presence of oxygen, however, there are only minor variations in the α coefficient, indicating that the yield of U_0 lesions is nearly independent of LET in aereated conditions. At very high LET there is no significant difference between the yield of U_0 lesions found in cells treated under either areated or hypoxic conditions. Thus, the presence of oxygen during irradiation will significantly increase the initial yield of U_0 lesions. It appears indeed that most, if not all, of the radiobiological oxygen effect relates to the initial production of U_0 radiolesions during the early radiation physics and chemistry phases.

In Figure 10B the values of the exponent $\varepsilon\phi$ are plotted as function of LET. In air as well as under hypoxic conditions there is rapid decrease noted in $\varepsilon\phi$ with increasing LET. Since ε is assumed to be constant, the measure of linear eurepair (ϕ) decreases rapidly with LET. We believe this is not because of a change in the value of λ or of k, but rather ϕ decreases because of the increased misrepair along individual ionizing particle tracks. The increased misrepair is caused by the physical closeness of U_0 lesions along the individual particle tracks. We visualize that a particle track with very high energy density has a high efficiency in producing DNA lesions

wherever the expanding core of this track intersects DNA. Calculations show that 10 to 20 U_0 lesions per ionizing track are likely in mammalian cell nuclei when the LET is greater than 100 keV/μm. At this LET the core diameter of a high speed heavy ion can be on the order of 10 to 20 nm (Chatterjee et al. 1973). This is the same order of magnitude as the size of nucleosomes, therefore, the damage might be extensive if a nucleosome is in the pathway of such a heavy-ion track.

We believe that the repair processes for heavy-ion-induced radiolesions are quite similar to the repair processes for x-ray-induced lesions. The key question is whether or not all lesions made by a single track can be eurepaired; if only one of the lesions is misrepaired it may cause a lethal effect. Further analysis of this problem is in progress.

Applications to the Radiation Responses of Synchronous Cell Populations

It is well known that mammalian cells exhibit variations in radiation sensitivity during the cell division cycle. Although a good deal of empirical material is available, this problem has received relatively little analysis from the point of view of quantitative mechanisms. The RMR model can give some information on the variations in sensitivity for producing U_0 lesions, and on changes in repair.

We have an experimental program to measure cell radiosensitivity in various stages of the cell cycle. The data given here should be regarded

as preliminary because we are still finding a good deal of variation in both the α and ϵ values from experiment to experiment. This may be because the process of synchronization interferes with the amount of the intracellular repair enzyme and with the chemical end groups that can modify radiosensitivity.

For x rays the most sensitive part of the cell cycle is mitosis, and we find ϵ, the repair ratio, to be very small for mitotic cells ($\epsilon = 0.02$) (Figure 11A). However, the yield of U_0 lesions (α) is comparable to and even somewhat lower than α at other cell phases. In the G and S phases, there is repair; much more in S ($\epsilon = 22.5$) than in G_1 ($\epsilon = 5.93$).

There is much less repair for any of the four cell phases irradiated with argon ions compared to the repair seen with x rays (Figure 11B). Survival curves obtained from cells irradiated with argon are almost purely exponential, and therefore it is more difficult to accurately determine the ϵ coefficient. However, ϵ is less than 1 for all of the cell cycle phases, and the yield of U lesions (α), all within 10% of each other. Interestingly, Sasaki and Okada (1979) found the initial yield of strand-breaks in mammalian cell DNA to be independent of the stage in the cell cycle.

We are only beginning to work on the synchrony problem. It appears that the rates of repair λ and k of equation (1) are both functions of cell age. It is likely that synchrony experiments will demonstrate a need for more detailed RMR models of the repair process than are given here.

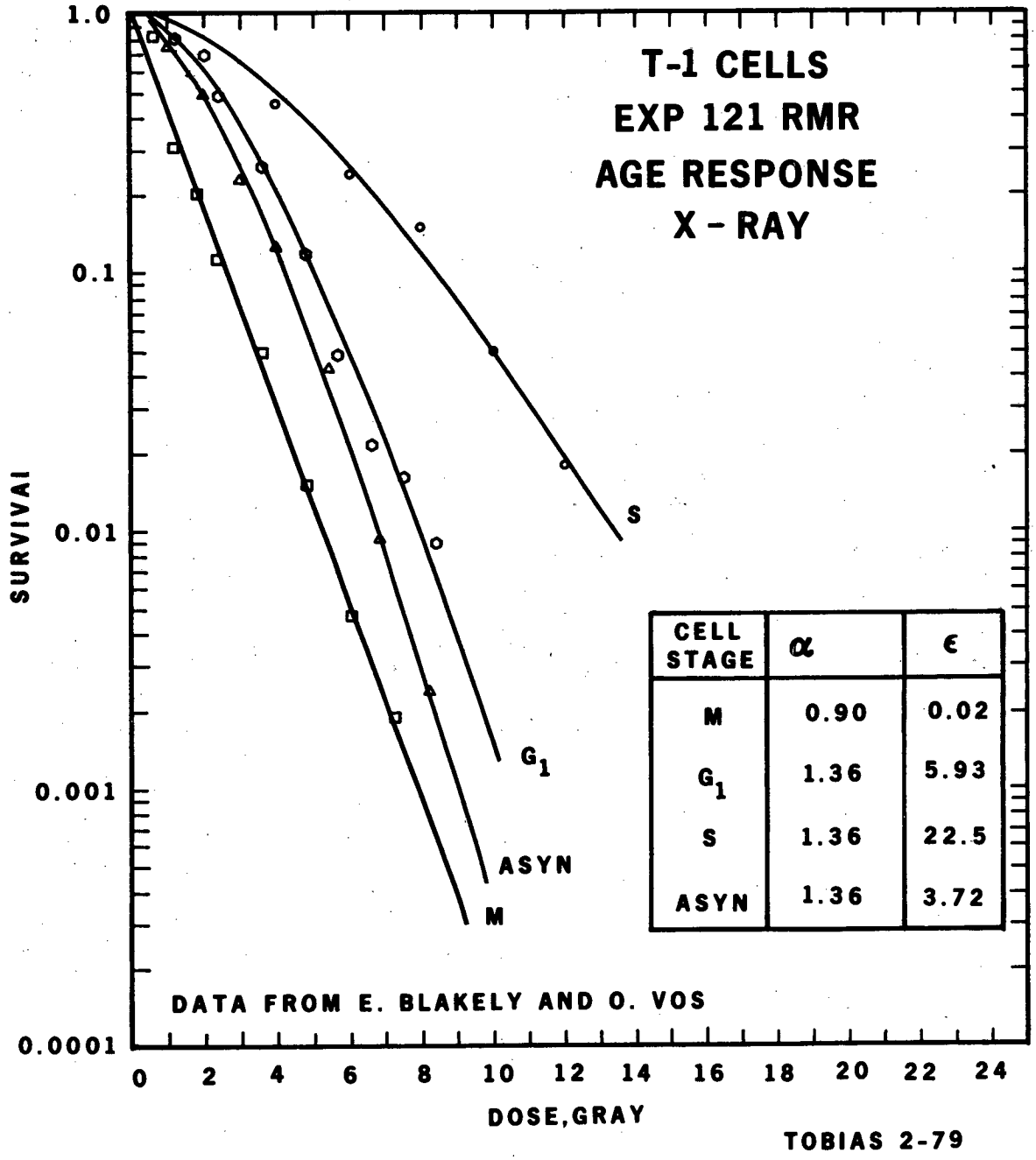

Figure 11A

T-1 cell survival curves obtained with 220 kV x rays for cells synchronized by mitotic shake-off. The data are from Vos et al. (1966) and Blakely et al.

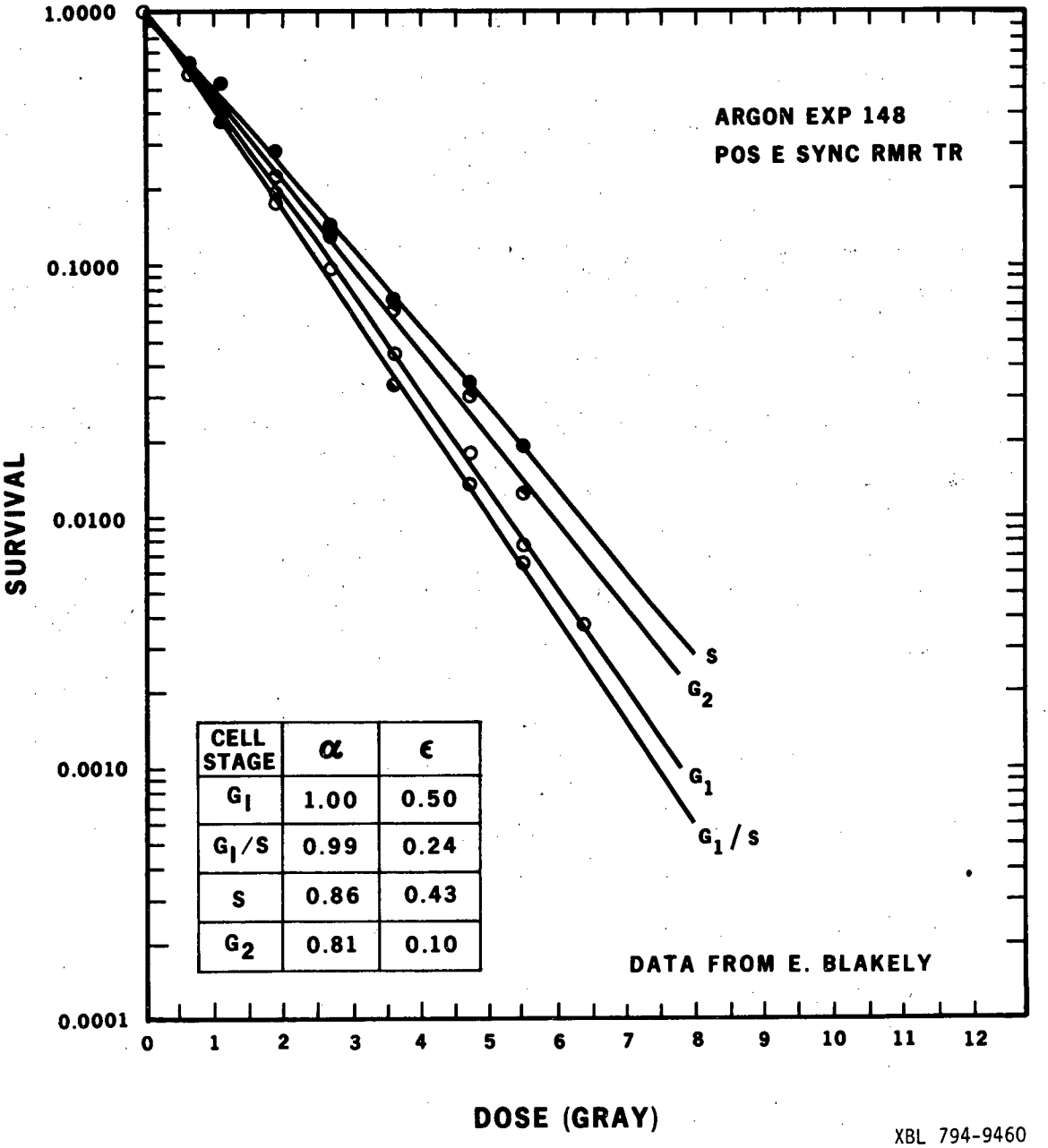

Figure 11B

T-1 cell survival curves obtained with argon ions of 0.35-cm residual range in water.

Case III of the RMR Model: Split Dose Experiments and Mixed Modalities

Equations (4), (5), and (6) are solutions of the basic RMR differential equation (1), and they give a detailed account of the time dependent quantities of U and R states following a single dose of radiation.

Assume that a dose D_1 is given first and that this is followed by a second dose D_2 of the same radiation after a time interval τ . (All relevant quantities of the first and second exposure are denoted by subscripts 1 and 2, respectively.) We propose to deal with this problem by introducing the concept of a "remnant lesion."

The symbol for remnant lesions, U_R , stands for the quantity of uncommitted lesions present per cell at time τ .

$$U_R(\tau) = \frac{U_1(0)e^{-\lambda\tau}}{1 + \frac{U_1(0)}{\varepsilon}(1 - e^{-\lambda\tau})} \tag{24}$$

If time scale t_2 begins with the second dose D_2 ($t_2 = t_1 - \tau$) we can write a solution to equation (1) by prescribing new boundary values:

$$U_2(0) = U_R + \alpha D_2, \quad \text{where } U_2(\infty) = 0 \tag{25}$$

Analogous to equations (4), (5), and (6) we have:

$$U_2(t_2) = \frac{(U_R + \alpha D_2)\, e^{-\lambda t_2}}{1 + \frac{(U_R + \alpha D_2)}{\varepsilon}(1 - e^{-\lambda t_2})} \tag{26}$$

$$R_L(t_2) = \varepsilon \ln \left[1 + \frac{(U_R + \alpha D_2)\ (1 - e^{-\lambda t_2})}{\varepsilon} \right] \tag{27}$$

$$R_Q(t_2) = \left[\frac{(U_R + \alpha D_2) \left(1 + \frac{(U_R + \alpha D_2)}{\varepsilon} \right) (1 - e^{-\lambda t_2})}{1 + \frac{(U_R + \alpha D_2)}{\varepsilon} \cdot (1 - e^{-\lambda t_2})} \right] \cdot$$

$$- \varepsilon \ln \left[1 + \frac{(U_R + \alpha D_2)\ (1 - e^{-\lambda t_2})}{\varepsilon} \right] \tag{28}$$

Calculation of the survival after two dose installments involves renormalization. We know that after time $t_1 = \tau$ of the first dose D_1 some cells are already committed to die since they have R_{QM} lesions. The number that still survive is $1 - L(\tau)$ of equation (12). The probability of survival after two doses, D_1 and D_2, separated by τ is:

$$S_{1,2} = [1 - L(\tau)]\ S(t_2) \tag{29}$$

$$S(D_1, D_2; \tau; t_2) = \exp\ [-U_R(\tau) - \alpha\{\gamma(\tau)\ D_1 + D_2\}] \cdot$$

$$\left[\left(1 + \frac{D_1(1 - e^{-\lambda t})}{\varepsilon} \right) \left(1 + \frac{(U_R + \alpha D_2)\ (1 - e^{-\lambda t_2})}{\varepsilon} \right) \right]^{\varepsilon} \tag{30}$$

where U_R is given in equation (24).

In Figure 12 we show a graphic analysis of the time dependence of the survival probabilities (S) and lethality probabilities (L) as functions of time. Three different conditions are compared: a single dose of 800 rad, a single dose of 400 rad, and two split doses of 400 rad each separated by a time interval τ . The area representing cells that have only U lesions is shaded. It is obvious from the figure that at τ there are cells with remnant lesions, and that the probability for U lesions increases stepwise with the addition of a second dose.

We have analyzed some split dose x-ray experiments on V79 hamster cells performed by Ngo (1978). In this type of split dose experiment it is necessary to evaluate the constants α and ϵ from single exposures in advance of the analog of the split-dose experiment. The usual experiment, shown in Figure 13, involves administering a preset single dose (D_1), and varying the size of the second dose (D_2), keeping τ constant. When this is done, we can evaluate the remnant lesions U_R and also the value of λ , the time rate constant of linear repair. When the values of ϵ and λ are known, the values for $k = \frac{\lambda}{\epsilon}$ can then be calculated.

Mixed Radiations

The equations given for split dose experiments can also be extended for mixed beams in special cases. One may initially ask the question whether or not two radiations can make uncommitted lesions of the same type for each other. This question can only be answered after extensive experimentation.

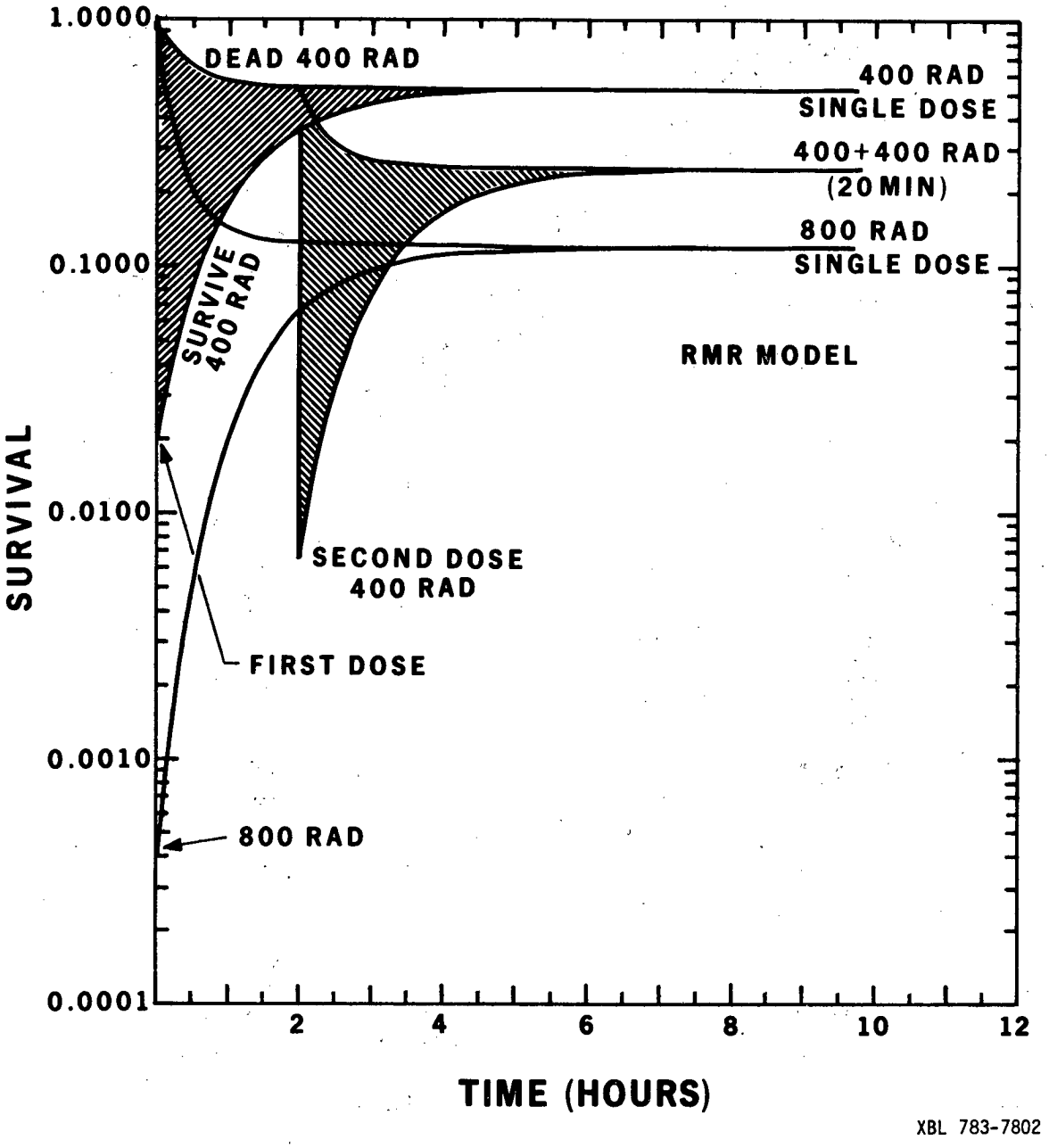

Figure 12

The time dependence of the functions S and 1-L are shown for a single dose of 400 rad, a single dose of 800 rad, and for split doses of 400 rad each separated by 20 minutes. The eventual observable survival levels are at large time values. Shaded areas correspond to uncommitted lesions. The time rates of change of these curves depend on the coefficient λ (equation 25). For this example, λ was assumed to be about three times greater than is actually the case in V-79 cells.

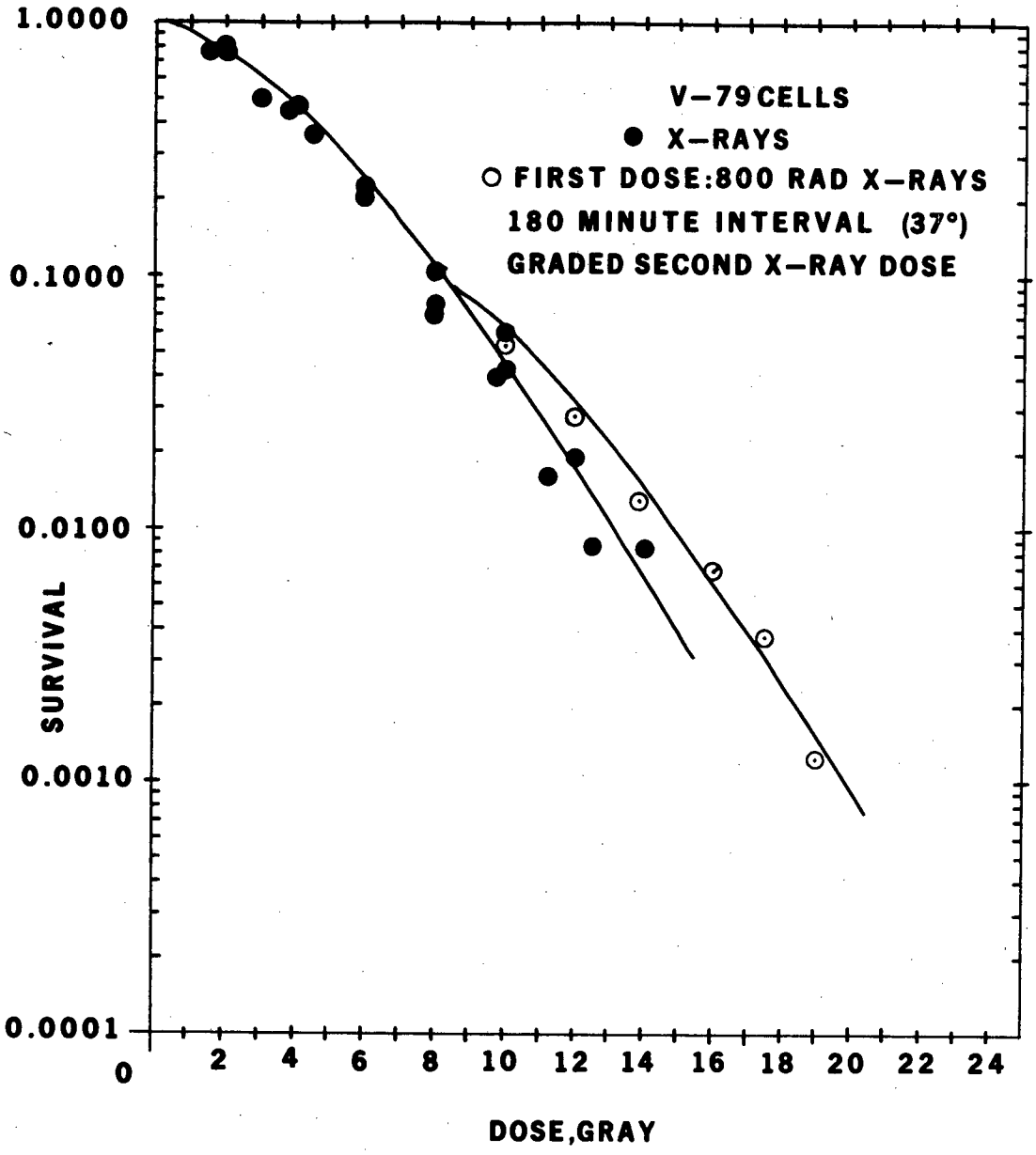

Figure 13

Split-dose experiment on V-79 cells with x rays; experimental data and RMR theory. ●, single dose, x rays; o, graded second doses; continuous lines, RMR model, following equation (30).

The usual experimental design starts with a dose of radiation 1 (e.g., neon ion beam), which is followed by a series of doses of radiation 2 (e.g., x rays). A time interval τ is set between the two exposures. The situation can be described by equations similar to equations (24) through (30), except that $\varepsilon_1 \neq \varepsilon_2$ and $\alpha_1 \neq \alpha_2$. (We do not show the explicit equations in this paper.)

Figure 14 graphs data from a mixed radiation experiment. Experiments like these established that an exposure to heavy ions (carbon, neon, or argon) produces remnant U lesions for x-rays and vice versa (Ngo 1978). We suspect, however, that the interaction between two modalities, and also between split doses of the same modality, is more complex than a mere overlap of sublethal lesions. This is illustrated by the fact that experimentally large doses of high LET neon or argon ions can under certain circumstances potentiate the effect for a second modality.

It appears quite likely that split dose and mixed modality exposures may uncover new repair mechanisms. It is of particular interest to extend this model to account for the effects from a variety of deleterious agents. Among the possible interactions are:

1. One modality makes entirely different molecular lesions than another modality and the repair mechanisms are also different.
2. One modality makes remnant lesions for another modality.
3. One modality may cause extranuclear effects (e.g., membrane damage) which may result in an alteration of the number of U lesions that can be produced by the other modality.

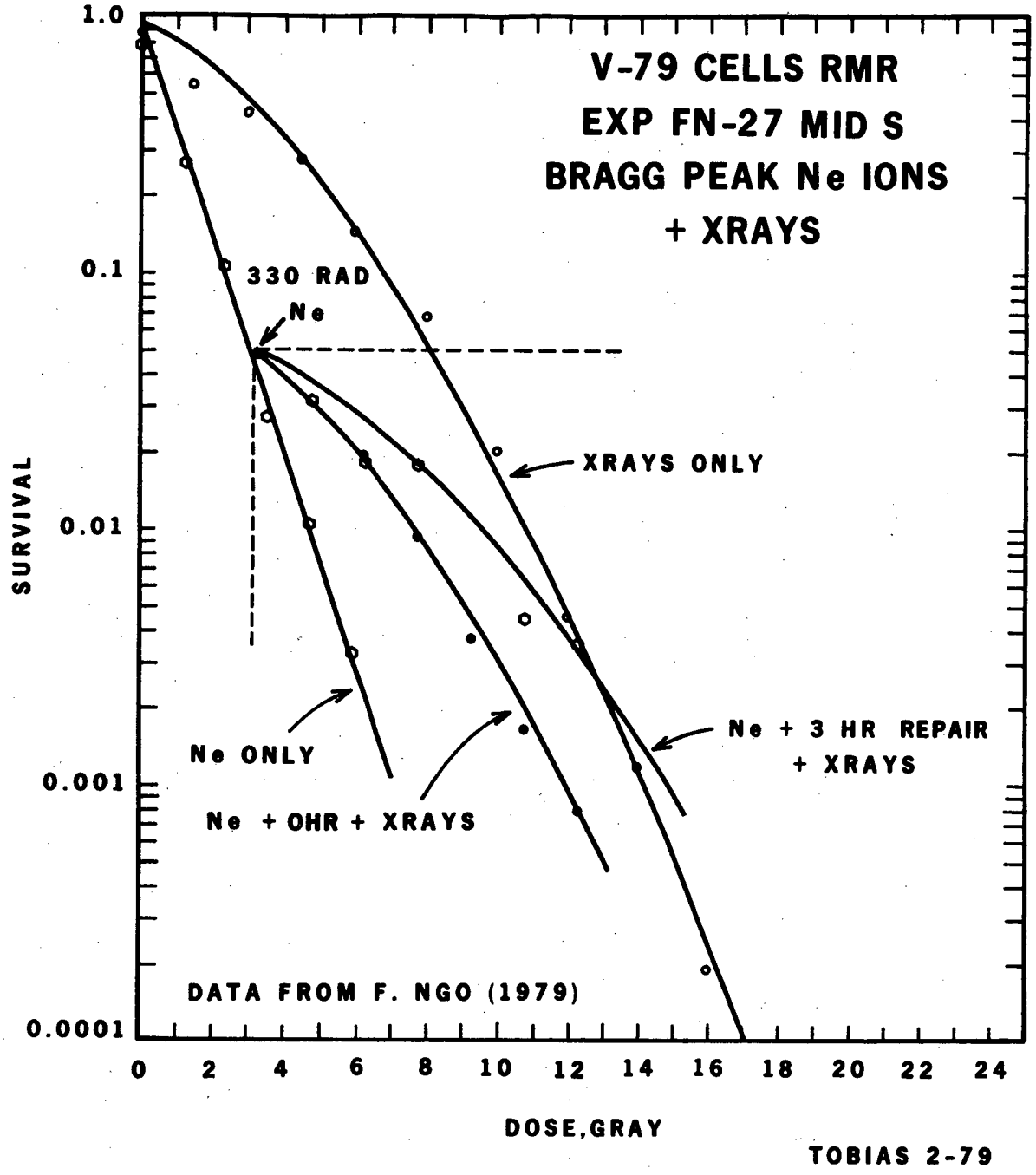

Figure 14

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Mixed beam experiments with V-79 cells. The curves shown are for x rays only, neon only, and neon followed by x rays. These experiments by F. Ngo et al. show that a previous dose of neon ions produced U lesions for x rays. After 330 rad neon dose, the "remnant" lesions for x rays correspond to about 210 rad of x rays; after waiting three hours at room temperature, the remnant lesions from 303 rad neon correspond to about 150 rad of x rays.

4. Exposure to a deleterious agent can impair or potentiate the repair mechanisms for the other modality.

The RMR model might be suitable for further expansion because it separates the dose-dependent production of radiolesions from the time-dependent repair, and because it has a built in "memory" for U_R lesions. Eventually the RMR model may be helpful in classifying a variety of lesions produced by a variety of agents.

The Role of Remnant Lesions and their Relationship to the Initial Slope of the Survival Curves at Low Doses

We may generalize the role of remnant lesions, U_R, which is defined in equation (24). These should also be related to radiobiological experiments on mammalian cells when a single dose is delivered. It appears that remnant U_R lesions might be present not only as a consequence of a previous dose of radiation, but also because of other, nonspecific events in the life of the cell that are not precisely understood at present. Remnant lesions can potentially lower the plating efficiency of cells and they are also able to alter the initial slope of the survival curve. Whereas it is quite likely that most of the plating efficiency variations in radiobiological experiments are not related to U lesions for ionizing radiations, the RMR model might be useful in unraveling some of the causes of variations in plating efficiency.

Consequently, using the RMR model there are at least three possible reasons for a finite initial slope of the survival curve:

1. a limited time (t_{max}) available for eurepair, usually the time to the first mitosis (see equations (10) and (17))
2. not all linear repair is eurepair; $\phi < 1$, see equation (8);
3. there are remnant U lesions from a previous dose of the same radiation, or a previous dose of another deleterious agent, see equations (24) and (30).

The problem of the initial slop of survival curves, and the initial slopes of mutation and transformation curves, is a serious one from the point of view of public health risk estimation. In some models, e.g., the dual action theory (Kellerer and Rossi 1972, 1978), a finite initial slope is firmly related to irreversible direct radiation injury. This is a rather different conclusion from the RMR model where at least three different classes of phenomena can alter the initial slope and where the survival curve of cells depends on their recent history of exposure to a variety of deleterious agents. We hope that the RMR model can be used to design crucial experiments that would point to the most important factors in the causation of low dose effects.

Case IV of the RMR Model: Mutations and Cell Transformation

There are several classes of mutations. The exact probability of producing a specific mutant or transformant depends on the number and types

of changes that must occur in DNA to produce the specific new structure. It is possible, and even likely, that a number of cell generations and several crucial events must occur to complete a mutation or transformation process. There is not sufficient room in this paper to discuss all factors affecting mutation rates, however, an example is given of how the mutation rate might be calculated.

Assume that mutations derive from misrepaired R states. According to our definitions (Table 1), R_{LM} and R_{QM} are states with abnormal genetic structures. If a cell with such states survives, there is a chance that its progeny will be mutants. Assuming a constant probability, we give a formula for the probability M (D,t) that a survivor is a mutant, based on survival equation (8).

$$M(D,t) = 1 - \left[1 + \frac{\alpha D (1 - e^{-\lambda t})}{\varepsilon}\right]^{-\Delta\phi\varepsilon} \qquad (31)$$

Here $\Delta\phi/\phi$ is the fraction of linear repair that results in a mutant. At very low doses, we obtain a linear relationship between dose and mutation:

$$M(D,t) = \Delta\phi \cdot \alpha (1 - e^{-\lambda t}) D \qquad (32)$$

whereas at high doses the mutant/survivor ratio is saturated. The mutant thus produced corresponds to a chromatid aberration, but whether or not it is

expressed depends on further genetic developments. The absolute number of mutations produced is nonlinear and has a maximum. Equation (31) generally fits the shapes of mutation yields obtained in heavy-ion enhancement of viral induced cell transformation studies by Yang et al. (1979).

Case V of the RMR Model: Radiation Effects on the Kinetics of the Repair Process

Inactivation of the Repair Mechanism

Because the dose- and time-dependent processes are handled separately, the RMR model is well suited for the study of the dose dependence of the kinetics of the repair process. In the survival expression equation (10) the coefficients λ, k, and ε were regarded as constants. For Case V we consider these coefficients to be dose-dependent quantities. We may regard λ as proportional to the available repair enzyme so that if the enzyme should be inactivated by a dose D of radiation, λ may change accordingly: $\lambda = \lambda(D)$. In Figure 15A we show theoretical examples of mammalian cell survival curves where the repair process is inactivated. We assumed that the inactivation kinetics are linear, along with the coefficient ψ.

$$\varepsilon(D) = \varepsilon_0 e^{-\psi D} \tag{33}$$

where $\varepsilon_0 = \varepsilon$ at zero dose and $\varepsilon(D)$ of equation (33) was substituted for ε in survival equation (7).

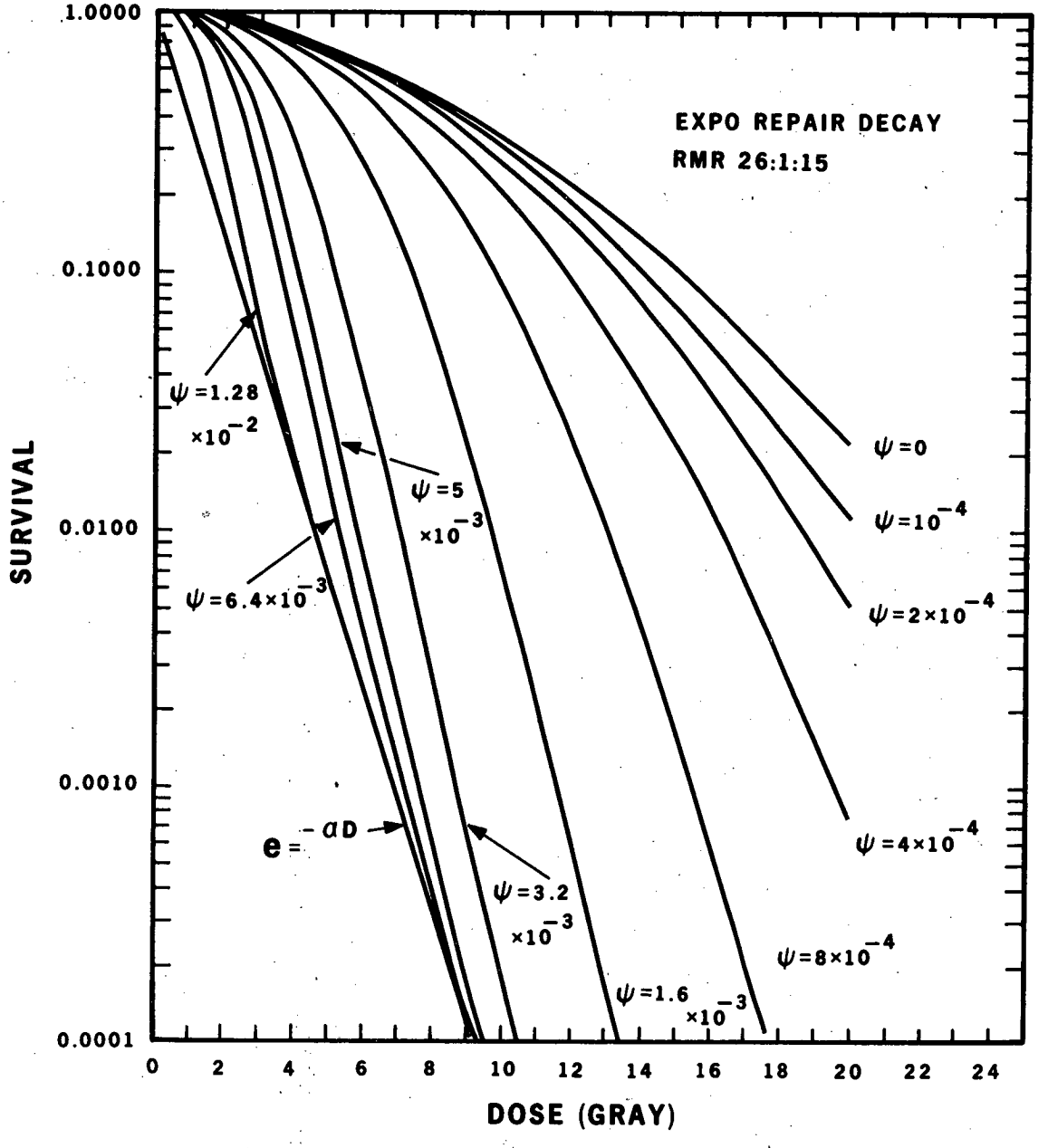

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Figure 15A

Theoretical survival curves, assuming dose-dependent inactivation of the repair mechanism. It was assumed that α (from equation 16) was constant. The repair ratio, ε, is a function of the dose delivered according to equation 33. The values of ψ on the graph are given in units of rad^{-1}. When ψ is about 10^{-3}, the survival curves have a quasi-exponential portion.

It is interesting to note that in Figure 15A the shape of the survival curves change from the usual continuously bending "RMR" form to shapes that behave like a simple exponential at medium high doses (8 to 16 Gy). This occurs when we assume that the repair mechanism is about ten times more resistant to ionizing radiation than the genome of the cells is to the production of U lesions. This type of dose-dependent behavior resembles a repair saturation curve such as proposed by Green and Burki (1972). However, at higher doses where the repair is completely inactivated the curves merge into the "repairless" curve $e^{-\alpha D}$. Thus, according to the RMR model, survival curves with shoulders and an exponential portion might be indicators of the presence of repair mechanisms that are damaged by a dose of radiation. It would also follow that repair would proceed more slowly after a large dose of radiation than following a small dose.

SOS Repair

The induction of repair by a dose of radiation is termed "SOS repair." Recently new types of repair of UV-induced lethal lesions were described in bacteri (Radman 1975; Devoret 1978; Witkin 1976), where a large dose of radiation induced a repair mechanism that was not present when a small dose of radiation was given. Howard and Cowie (1978) also showed SOS repair in algae. As far as we know, the genes controlling repair enzymes in mammalian cells are constitutive; however, more investigations appear necessary.

The RMR model is quite suitable for the study of rate processes involved in SOS repair. In Figure 15B we show survival probabilities for SOS repair when an appropriate function of dose is inserted in the ϵ parameter of equation (17).

Case VI of the RMR Model: Dose-Rate Effects

In all of the preceding cases it was tacitly assumed that the dose was delivered so fast to the cells that exposure to radiation was complete before the repair processes were underway. In this section, we shall demonstrate that the RMR model can be used for modeling survival at low as well as high dose rates. At very high dose rates (e.g., > 10^5 Gy/min), the RMR model is probably not valid.

Assume a constant dose rate $\dot{D}$. Uncommitted lesions accrue at the constant rate $a(\dot{D})$ in accordance with equations (13) through (15). In our example, we choose $a(\dot{D}) = \alpha\dot{D}$ from equation (14). The differential equation for uncommitted lesions per cell which is analagous to equation (1) is:

$$\frac{dU}{dt} = a - \lambda U - kU^2 \tag{34}$$

Integrating and using the quantities R_L and R_Q defined in equations (2) and (3) we have

$$U + R_L + R_Q = at \tag{35}$$

$$S = \frac{\left[1 + \frac{\alpha D}{\epsilon}\left(1 - e^{-\lambda T}\right)\right]^{\epsilon}}{e^{\alpha D}}$$

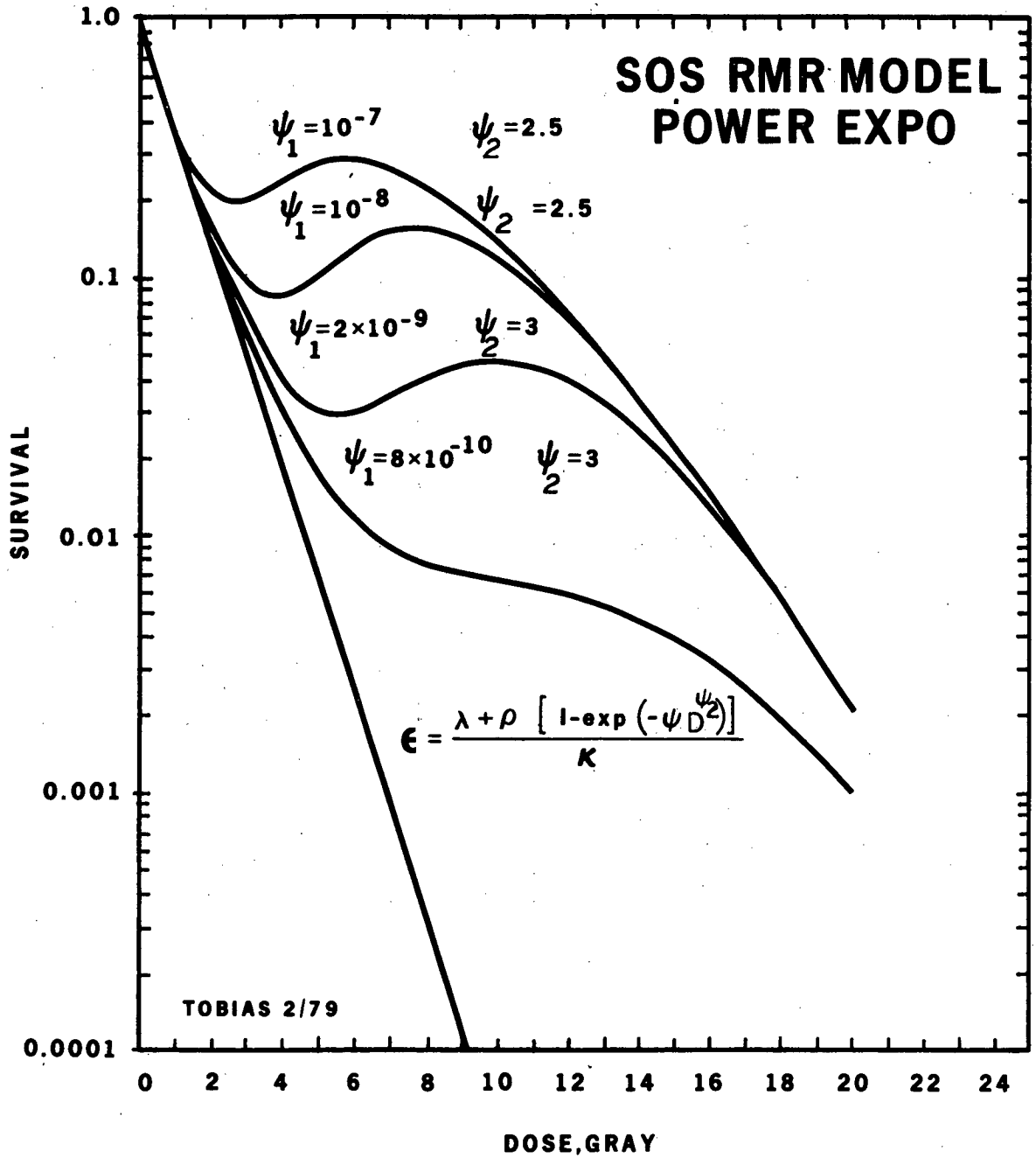

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Figure 15B

SOS survival curves generated by the RMR model. To do this, we assumed a dose dependence of ε, as shown by the equations on the graph.

Figure 12

The time dependence of the functions S and 1-L are shown for a single dose of 400 rad, a single dose of 800 rad, and for split doses of 400 rad each separated by 20 minutes. The eventual observable survival levels are at large time values. Shaded areas correspond to uncommitted lesions. The time rates of change of these curves depend on the coefficient λ (equation 25). For this example, λ was assumed to be about three times greater than is actually the case in V-79 cells.

Figure 13

Split-dose experiment on V-79 cells with x rays; experimental data and RMR theory: •, single dose, x rays; o, graded second doses; continuous lines, RMR model, following equation (30).

Figure 14

Mixed beam experiments with V-79 cells. The curves shown are for x rays only, neon only, and neon followed by x rays. These experiments by F. Ngo et al. show that a previous dose of neon ions produced U lesions for x rays. After 330 rad neon dose, the "remnant" lesions for x rays correspond to about 210 rad of x rays; after waiting three hours at room temperature, the remnant lesions from 303 rad neon correspond to about 150 rad of x rays.

Figure 15A

Theoretical survival curves, assuming dose-dependent inactivation of the repair mechanism. It was assumed that α (from equation 16) was constant. The repair ratio, ε, is a function of the dose delivered according to equation 33. The values of ψ on the graph are given in units of rad^{-1}. When ψ is about 10^{-3}, the survival curves have a quasi-exponential portion.

With boundary values of $U(0) = 0$ and $U(\infty) = U$, we can solve for $U(t)$:

$$U(t) = U_\infty \cdot \gamma_a(t) \tag{36}$$

where: $U_\infty = -\varepsilon/2 + \left((\varepsilon/2)^2 + (a/k)\right)^{\frac{1}{2}}$

$$\lambda_a = \lambda + 2kU_\infty \quad ; \quad \varepsilon_a = \lambda a/k$$

and: $$\gamma_a(t) = \frac{\left(1 - (U_\infty/\varepsilon_a)\right)\left(1 - e^{-\lambda_a t}\right)}{\left(1 - (U_\infty/\varepsilon_a)\right)\left(1 - e^{-\lambda_a t}\right)}$$

If we proceed in a manner similar to equations (1) through (6) and (17), we can calculate time-dependent probabilities of survival $S(a,t)$ and lethality $L(a,t)$:

$$S(a,t) = \left[1 - (U_\infty/\varepsilon_a)\left(1 - e^{-\lambda_a t}\right)\right]^{\varepsilon} \exp\left(\lambda_a U_\infty - a\right)t \tag{37}$$

$$1 - L(a,t) = \left[1 - (U_\infty/\varepsilon_a)\left(1 - e^{-\lambda_a t}\right)\right]^{\varepsilon} \exp\left[\left(\gamma_a U_\infty + (\lambda_a U_\infty - a\right)t\right] \tag{38}$$

If radiation with a constant dose rate D is delivered for a time period τ, a remnant lesion of $\gamma_a(\tau)U$ will be present at the end of period τ. Repair will continue with the further passage of time.

$$S_t = S(a,\tau)\left(1 + \frac{\gamma_a(\tau)U_\infty}{\varepsilon}\left(1 - e^{-\lambda(t-\tau)}\right)\right)^{\varepsilon} \cdot e^{-\gamma_a(\tau)U_\infty} \qquad (39)$$

where $S(a,\tau)$ is taken from equation (37)

In Figure 16 we have plotted the functions S(a,t) and S(t) for widely varying dose rates, but each with the same total dose. The survival depends on dose rate if the dose is delivered in about the same lenth of time as the half time for repair.

The example for a typical mammalian cell system assumes that the half life for repair is longer than one hour. In this example, the dose can be delivered in any time interval, from 0 to about 10 minutes, without appreciably affecting survival.

Kaplan (1974), found "fast" repair processes in bacterial DNA which were on the order of one minute, and a similar rate of repair process was found recently by Braby and Roesch (1978), in the algae chlamydomonas, which was irradiated continously . The model described here is suitable for calculating survival for repair rates that are $\lambda \simeq 10^3$/sec or smaller, but in mammalian cells we have no evidence for such high repair rates.

It is important for the validity of the RMR model that the rate dependent events involving fast radical chemistry do not significantly overlap in time with the enzymatic repair processes we discuss here. From the work of Epp

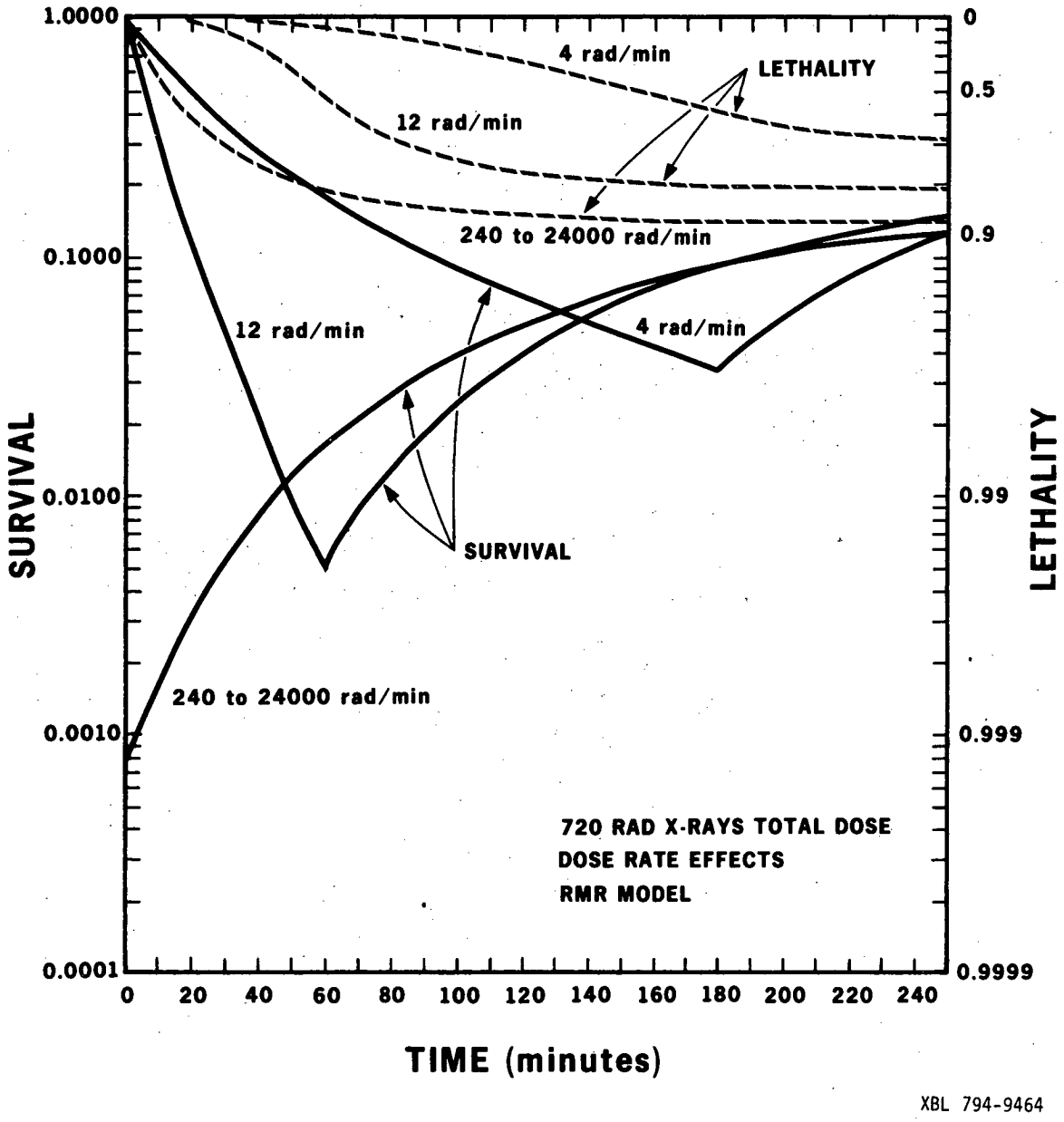

Figure 16

Prediction of the RMR model for continuous dose rate exposure. S and 1-L curves are shown for 4 rad/min and 12 rad/min doses. Above 10,000 rad/min the model predicts no dose-rate effect. Note that the U lesions accumulate with time while the radiation is "on."

et al. on bacteria (1968; Michaels et al. 1978), it appears that very high dose rates (high enough so that the entire dose is delivered in about 10^{-4} seconds) are necessary to modify the oxygen effect. Anoxic radiosensitivity, on the other hand, appears to be insensitive to high dose rates up to 10^{12} rad/min. Todd et al. performed very high dose rate experiments in electron beams with human kidney cells (1968). Their work, reinterpreted recently by Braby and Roesch (1978), is indicative of a small dose rate effect above 10^{11} rad/min. Such results essentially confirm the assumptions of our model: that dose and time dependent physical-chemical interactions are essentially over in less than 10^{-3} seconds.

Continuous Background Radiation and the Initial Slope of Survival Curves

Background radiation, according to equations (34) through (38), continuously delivers new U lesions to cells and, in spite of repair, there are always some U lesions present in DNA under these conditions.

The background radiation caused by cosmic rays, and radioactivity found in the tissues and the environment, continuously produce lesions in DNA nucleoprotein. Misrepair of such lesions is also bound to occur, resulting in occasional lethal effects. As a result of the presence of remnant lesions from background radiation, the RMR model predicts that the initial dose-dependent slope of survival curves following acute low- or high-LET radiation is never exactly zero but always has a finite, albeit, possibly very low, value.

SUMMARY

A new model is presented for cell survival, lethality and mutation caused by ionizing radiations: the repair misrepair model (RMR). We have shown that the fast events of physical energy transfer and of radiation chemistry are largely complete before the enzymes of a living cell can recognize relevant macromolecular processes and before biochemical repair processes are under way. This allows the model to be separated into dose-dependent and time-dependent processes; the shapes of the survival curves depend on both. Initial macromolecular lesions are regarded as uncommitted because the eventual fate of cells remains uncertain for some time after exposure. The enzymatic repair processes yield either eurepaired states or misrepaired states with altered structures. Survival is a result of competition between eurepair and misrepair. A fraction of misrepair leads to lethality; other misrepair fractions produce mutants. The general features of the misrepair process are analogous to chromosome rejoinings.

RMR survival kinetics have been applied to a variety of radiobiological processes including the analysis of repair-deficient mutants, the cell age response, the effects of accelerated heavy ions, split dose survival from mixed modalities, and induction of mutations. The model provides a flexible framework for testing mechanisms of the biological effects of ionizing radiations and of other deleterious agents. Dose-rate effects have also been modeled. Work is in progress to adapt it to such processes as repair inactivation and SOS repair.

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LIST OF SYMBOLS

t = time

D = dose (usually delivered at $t = o$); $\dot{D} = \frac{dD}{dt}$ = dose rate

$S(t)$ = probability of survival

$L(t)$ = probability of lethality

$M(t)$ = probability of mutation

U = number of uncommitted lesions/cell

$U(o) = U_o$ initial number of U lesions

U_R = remnant U lesion (at time t)

R = repair states

R_L = yield of a linear repair processes

R_Q = yield of a repair process involving interaction between pairs of U lesions

λ = coefficient of linear repair

k = coefficient of quadratic repair

ϵ = repair ratio (λ / k)

ϕ = probability that linear repair = eurepair

δ = probability that quadratic repair = eurepair

α = yield of U lesions per unit dose

τ = time interval separating two dose installments

T = time constraint = $(1 - e^{-\lambda t})$

m = number of hits in conventional target theory

x, y = constant coefficients in the LQ model

$a(\dot{D})$ = rate of production of U lesions

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