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Queueing Models of Potentially Lethal Damage Repair in Irradiated Cells

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ABSTRACT

Some of the ideas arising in queueing theory are applied to describe the repair mechanisms responsible for recovery of cells from potentially lethal radiation damage. Two alternative versions are presented of a queueing model of damage repair after a single dose of irradiation. The first version represents a linear misrepair model, and the second invokes the idea of spontaneous lesion fixation. They are pieced together in the third model, allowing for both mechanisms. The consistency of the proposed models with published experimental data is tested.

1. INTRODUCTION

The repair of potentially lethal radiation damage manifests itself in experiments with a delayed explanation (subculture) of cells irradiated in the stationary stage of their growth in vitro. When cultured cells are seeded at low concentrations they multiply exponentially for several days. Eventually, however, the cultures enter the stationary phase of growth, which is characterized by a constant cell number and a low proliferation activity. Either the irradiated stationary cultures are subcultured immediately to assay for the surviving fraction, or the subculturing is delayed for several hours. If allowed to remain in the stationary phase of growth for a while after irradiation, the cells repair some of the potentially lethal damage, resulting in enhanced cell survival as the delay time increases. With the passage of time this process reaches its

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saturation, which may be attributable to the completion of the recovery of cells from radiation damage. Relevant examples are discussed in this paper.

This phenomenon is of considerable significance to cancer radiotherapy. Experiments with nine lines of human tumor cells cultivated in vitro [1,2] have shown that in cells obtained from radiocurable tumors the potentially lethal damage repair in a stationary culture is much less pronounced than in those from neoplasms that are not radiocurable. This observation implies that intensive repair of potentially lethal lesions by resting tumor cells in the interval between consecutive dose fractions may account for the failure to ensure local control of a nonradiocurable tumor with certain regimes of fractionated irradiation. It is also known that hypoxic tumor cells recover more effectively from potentially lethal damage than their well-oxygenated counterparts [3].

Starting from the seminal paper of Clifford [4], and even earlier, there have been many attempts at a comprehensive theory of radiation damage repair. Over many years the idea of a saturable repair system has been considered a good alternative to the incorporation of nonlinear interactions between radiation-induced lesions in cell survival models. Goodhead [5] adduced experimental facts supporting the following premises, which might serve as a basis for mathematical modeling of radiation effect upon a cell:

- Radiation damage occurs at short distances from the sites of absorption of small amounts of energy—the predominant role in producing damage being played by the single-track mechanism.
- The number of lesions is proportional to the radiation dose.
- Damage can be reduced by the intracellular repair system, whose efficiency drops as radiation dose increases.

Alper [6] also came to the conclusion that radiation damage occurs in accordance with the one-hit mechanism and that relatively low sensitivity of cells in the event of small doses is associated not with the multiplicity of targets or the interaction between separate lesions but with the existence of a mechanism of enzymatic repair of lesions. Alper stressed that high doses of radiation suppress the repair mechanism's functioning, apparently due to the exhaustion of the biochemical factors (repair enzymes) involved in it. Alper noted that only in the case of an exponential survival curve is there a reason to believe that either the mechanism does not function at all or it maintains equal efficiency over the entire range of doses. According to Alper [6], this idea was first set forth by Powers [7].

A number of models have been proposed to formalize the idea mathematically [8–12]. The assumption that a cell has a limited repair

capacity is the distinctive feature of the models of the saturable repair enzyme type. The presently more fashionable repair–misrepair model [13–19] relates radiation-induced cell death to the functioning of error-prone repair mechanisms. There is no insurmountable contradiction between the two biologically plausible models. It seems likely that the advantages of both approaches could be combined in a parsimonious model formulated in terms of queueing theory. Such a formulation seems to be natural from the biological standpoint. It is widely believed that the critical cellular target for the cell-killing effect of radiation is DNA. Damage inflicted on DNA by irradiation is represented by DNA breaks and modified bases. Unless correctly repaired, such lesions may result in cell death. Repair of the DNA template is a complex gene-controlled process that involves inducible enzymes. As one example, the excision repair of the UV-induced thymidine dimers is controlled by at least seven gene products [20]. Hanawalt [21] conjectured that the excision repair mechanisms are processive, being coupled to transcription at the nuclear matrix. Thus the repair system is likely to be built up from a number of discrete repair units, or reparons [22], that can be viewed as *servers* intended to maintain the integrity of DNA. The possibility of thinking of the repair mechanisms as a queueing system was preliminarily explored in the context of radiation-induced carcinogenesis [23,24].

When applied to the analysis of cell survival data, a pertinent queueing model of the repair system offers two distinct advantages:

1. It allows the results of data analysis to be interpreted in terms of parameters having a clear biological meaning.
2. It provides ready-made methods [25] for computing some substantive characteristics of the repair system other than those estimated from experimental data. The relative capacity of the queueing system considered in Section 2.3 of the present paper falls into this category.

With some reasonable assumptions, the queueing formulations of the cell repair model are computationally tractable, providing a useful underpinning for the analysis of real data.

In this paper three versions of a queueing model of the repair system are considered with special reference to experimental data on potentially lethal damage repair in stationary cell cultures. The first model allows for misrepair of radiation-induced lesions due to repair errors that occur with a constant probability. To retain the model tractability, no attempt has been made to construct a queueing counterpart of the pairwise misrepair model [16,19]. This might be a good challenge for further research. The second model presumes the existence of a mechanism of spontaneous lesion fixation postulated earlier by other authors

[26]. The third version combines the ideas of both approaches. Each of the models leaves room for different causes of cell death. The usefulness of the proposed approach is illustrated with an application to published data on the recovery of cultured cells from potentially lethal radiation damage.

2. THE MODELS

Common to all of the models considered below are the following basic assumptions:

Assumption 1. The immediate consequence of irradiation with dose D is a formation of primary lesions in irradiated cells. In accordance with the "hit-and-target" principle [27], the number of such lesions, ν_0, is a Poisson random variable with expectation θD, that is,

$$\Pr(\nu_0 = N) = \frac{(\theta D)^N}{N!} e^{-\theta D}. \tag{1}$$

The parameter θ has the meaning of the expected number of lesions per unit dose, thereby characterizing radiosensitivity of a cell.

Assumption 2. The primary lesions are postulated to be subject to repair processes, and the repair results in lesion elimination. Given that single-dose irradiation has resulted in the formation of N lesions, the functioning of the repair system is modeled as a pure death process with m servers and a queue. The service is exponential with intensity μ. The usual independence assumptions are accepted. The queue discipline is selection for service in random order. The rationale for the latter assumption is that DNA lesions occur randomly within the cell genome in accordance with the physical properties of ionizing radiations. There is experimental evidence that the excision repair of pyrimidine dimers and some other lesions is nonrandom, exhibiting a preference for actively transcribed DNA sequences [21]. In the models developed here, this heterogeneity in repair is ignored lest the models become too cumbersome. This, however, is of no serious concern because the lesions responsible for cell death are believed to be associated with active regions of the genome.

2.1. *MODEL* 1

The model structure is shown in Figure 1. In the act of serving a lesion an error may occur with probability β. This event is known as misrepair and involves the lesion fixation that ultimately leads to cell death. The explantation of cells also results in the fixation of certain

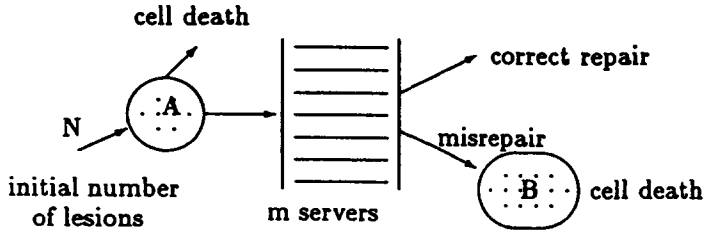

FIG. 1. Diagram of Model 1.

lesions. Some of the primary lesions may happen to remain unrepaired by the time of subculturing. After having been fixed at the time of subculturing, such lesions also cause cell death. The unrepaired and fixed lesions will be designated A lesions to distinguish them from the misrepaired ones or B lesions. The death of a cell is caused by a single lesion of either of the two types; that is, the one-hit mechanism of cell survival is assumed. The following two possibilities are worth considering in the context of this classification.

Case 1. The lesions of type A are those waiting for service at the time of subculturing.

Case 2. Every unrepaired lesion, be it under service or not, is included in a pool of A lesions.

As the time after irradiation increases, the cells have a better chance to repair A lesions, thereby reducing their numbers by the moment of subculturing. In parallel with the elimination of A lesions in a cell, there is the accumulation of B lesions, which gives an explanation for the fact that the enhancement of cell survival reaches its saturation point.

The problem is to give an expression for the cell survival probability $S(D;t)$ as a function of the irradiation dose D and the time of subculturing t, provided the cell is exposed to radiation at time $t = 0$. A service system is described completely by the probabilities of each possible state of the system. Such states can be numbered in accordance with the number of demands in the system. Referring to the pure death process with m-channel service, the probability $P_i(t)$ of exactly i demands being present (waiting in the queue or being serviced) in the system at time t satisfies the following system of differential equations:

$$\frac{dP_0(t)}{dt} = \mu P_1(t), \tag{2a}$$

$$\frac{dP_i(t)}{dt} = -i\mu P_i(t) + (i+1)\mu P_{i+1}(t) \qquad \text{for } 0 < i < m, \tag{2b}$$

$$\frac{dP_{m+r}(t)}{dt} = -\mu m P_{m+r}(t) + \mu m P_{m+r+1}(t) \qquad \text{for } 0 \leqslant r < N-m, \tag{2c}$$

$$\frac{dP_N(t)}{dt} = -\mu m P_N(t), \tag{2d}$$

given $N > m$. The initial conditions for (2) are specified as $P_i(0) = 0$ for $i < N$, and $P_N(0) = 1$.

It follows from Equation (2d) that

$$P_N(t) = e^{-\mu m t},$$

and by the backward substitution for unknowns in (2) we get

$$P_{m+r}(t) = \frac{(\mu m t)^{N-m-r}}{(N-m-r)!} e^{-\mu m t} \qquad \text{for } 0 \leqslant r \leqslant N-m. \tag{3}$$

To find a solution of (2) for $0 \leqslant i < m$, we introduce the generating function,

$$G(t;s) = \sum_{i=0}^{m} P_i(t) s^i.$$

Multiplying the first $m+1$ equations of system (2) by s^i and summing them, we obtain

$$\frac{d}{dt} \sum_{i=0}^{m} P_i(t) s^i = -\mu \sum_{i=0}^{m} i P_i(t) s^i + \mu \sum_{i=0}^{m-1} (i+1) P_{i+1}(t) s^i + m\mu P_{m+1}(t) s^m,$$

which is equivalent to the partial differential equation

$$\frac{\partial G}{\partial t} = -\mu(s-1)\frac{\partial G}{\partial s} + m\mu P_{m+1}(t) s^m. \tag{4}$$

The solution of (4), satisfying the initial condition $G(0;s) = 0$, is of the form

$$G(t;s) = m\mu \int_0^t \left[(s-1)e^{-\mu(t-u)} + 1\right]^m P_{m+1}(u)\, du.$$

Using the fact that

$$P_i(t) = \frac{1}{i!} \frac{\partial^i G(t;s)}{\partial s^i}\bigg|_{s=0}, \qquad i = 0,1,\ldots,m,$$

we obtain

$$P_i(t) = m\mu \binom{m}{i} \int_0^t [1 - e^{-\mu(t-u)}]^{m-i} e^{-i\mu(t-u)} P_{m+1}(u)\, du \tag{5}$$

for $0 \leqslant i \leqslant m < N$. In particular, we have

$$P_0(t) = m\mu \int_0^t [1 - e^{-\mu(t-u)}]^m P_{m+1}(u)\, du. \tag{6}$$

If $m \geqslant N$, the solution is given by the binomial distribution

$$P_i(t) = \binom{N}{i} (1 - e^{-\mu t})^{N-i} e^{-i\mu t}, \qquad 0 \leqslant i \leqslant N. \tag{7}$$

Remark 1. One of the reviewers pointed out a somewhat simpler way of deriving formula (5). Let $X(t)$ be the number of demands in the system at time t. The function $X(t)$ decreases from N as long as $X(t) \geqslant m+1$. The probability that $X(t)$ reaches m at time u is $m\mu P_{m+1}(u)\, du$. Conditional on this event, the probability that $i \leqslant m$ lesions remain at $t > u$ is a binomial of the form

$$\binom{m}{i} [1 - e^{-\mu(t-u)}]^{m-i} e^{-i\mu(t-u)},$$

which, upon undergoing a compounding procedure, immediately gives formula (5).

Now the causes of cell death can be specified explicitly for the two cases mentioned above.

Model 1.1. In this case we assume that the subculture does not stop the repair process and the subcultured cells have enough time for its completion. The conditional survival probability, given the time of subculturing t and the number of primary lesions N, is equal to

$$F(N,t) = \begin{cases} (1-\beta)^N \sum_{k=0}^{m} P_k(t) & \text{if } N > m, \\ (1-\beta)^N & \text{if } 0 \leqslant N \leqslant m. \end{cases} \tag{8}$$

If $N > m$, it follows from (5) that

$$\sum_{k=0}^{m} P_k(t) = m\mu \sum_{k=0}^{m} \binom{m}{k} \int_0^t [1 - e^{-\mu(t-u)}]^{m-k} e^{-k\mu(t-u)} P_{m+1}(u)\, du$$

$$= m\mu \int_0^t P_{m+1}(u)\, du, \tag{9}$$

where $P_{m+1}(u)$ is given by (3). Substituting (9) in (8),

$$F(N,t) = \begin{cases} m\mu(1-\beta)^N \int_0^t P_{m+1}(u)\, du, & N > m, \\ (1-\beta)^N, & 0 \leqslant N \leqslant m. \end{cases}$$

Taking formula (3) and the fact that $\sum_{k=0}^{N} P_k(t) = 1$ into account, the conditional probability $F(N,t)$ can be represented as

$$F(N,t) = \begin{cases} (1-\beta)^N \left(1 - \sum_{k=0}^{N-m-1} \frac{(\mu m t)^k}{k!} e^{-\mu m t}\right) & \text{if } N > m, \\ (1-\beta)^N & \text{if } 0 \leqslant N \leqslant m, \end{cases} \tag{10}$$

and the overall survival probability is given by

$$S(D;t) = \sum_{N=0}^{\infty} \frac{(\theta D)^N}{N!} e^{-\theta D} F(N,t), \qquad t > 0. \tag{11}$$

If $t = 0$ (no delay in subculturing) the dose–effect relationship (11) acquires the form

$$S(D;0) = \sum_{N=0}^{m} \frac{[\theta D(1-\beta)]^N}{N!} e^{-\theta D}. \tag{12}$$

It is instructive to consider what happens when $t \to \infty$. Since the series in (11) converges uniformly with respect to $t \geqslant 0$, we may write

$$\lim_{t \to \infty} S(D;t) = \sum_{N=0}^{m} (1-\beta)^N \frac{(\theta D)^N}{N!} e^{-\theta D}$$

$$+ m\mu \sum_{N=m+1}^{\infty} (1-\beta)^N \frac{(\theta D)^N}{N!} e^{-\theta D} \int_0^{\infty} P_{m+1}(u)\, du.$$

Using (3), we may readily check that

$$m\mu\int_0^\infty P_{m+1}(u)\,du = 1.$$

Consequently,

$$\lim_{t\to\infty} S(D;t) = \sum_{N=0}^{m} (1-\beta)^N \frac{(\theta D)^N}{N!} e^{-\theta D} = e^{-\beta\theta D}. \tag{13}$$

If we assume, as the other extreme, that the explantation of cells stops the repair process completely but the lesions whose repair has been started by that time are not responsible for cell death, a modified version of the model is easy to obtain. Instead of (8) we will have

$$F(N,t) = \begin{cases} \sum_{k=0}^{m} P_k(t)(1-\beta)^{N-k}, & N > m, \\ \sum_{k=0}^{N} P_k(t)(1-\beta)^{N-k}, & 0 \leqslant N \leqslant m. \end{cases}$$

Let $N > m$; then

$$\begin{aligned} F(N,t) &= (1-\beta)^{N-m} m\mu \int_0^t \sum_{k=0}^{m} \binom{m}{k} [1-e^{-\mu(t-u)}]^{m-k} (1-\beta)^{m-k} \\ &\quad \times e^{-k\mu(t-u)} P_{m+1}(u)\,du \\ &= (1-\beta)^{N-m} m\mu \int_0^t [1-\beta(1-e^{-\mu(t-u)})]^m P_{m+1}(u)\,du. \end{aligned}$$

For $0 < N \leqslant m$,

$$\begin{aligned} F(N,t) &= \sum_{k=0}^{N} \binom{N}{k} (1-e^{-\mu t})^{N-k} (1-\beta)^{N-k} e^{-k\mu t} \\ &= [1-\beta(1-e^{-\mu t})]^N. \end{aligned}$$

In the case of immediate subculturing,

$$S(D;0) = \sum_{N=0}^{m} \frac{(\theta D)^N}{N!} e^{-\theta D}, \tag{14}$$

which coincides with a multihit–one target model of radiation cell survival [27]. Formula (13) still stands for the limiting behavior of $S(D;t)$ for $t \to \infty$.

Remark 2. A reasonable compromise between the two versions of Model 1.1 can be worked out but at the cost of an increase in the number of unknown parameters. In doing so, we introduce a random time Y, assuming that any lesion that has been under service longer than Y by the moment t continues on its way to the complete repair, otherwise it is fixed by the explantation procedure. The misrepair may occur with probability β. The random variable Y is exponentially distributed with parameter η. Let $P_{n,k}(t)$ stand for the probability that the number of lesions left in the system at the moment t is equal to n and there are exactly k lesions whose time under service is less than Y. If $N \geqslant m$, the probabilities $P_{n,k}(t)$ satisfy the system of differential equations

$$\frac{dP_{n,k}(t)}{dt} = \mu(m-k+1)P_{n+1,k-1}(t) + \mu k P_{n+1,k}(t) + \eta(k+1)P_{n,k+1}(t) - (m\mu + k\eta)P_{n,k}(t)$$

$$\text{for } m \leqslant n \leqslant N;\ 0 \leqslant k \leqslant m;$$

$$\frac{dP_{n,k}(t)}{dt} = \mu(n-k+1)P_{n+1,k}(t) + \mu(k+1)P_{n+1,k+1}(t) + \eta(k+1)P_{n,k+1}(t) - (n\mu + k\eta)P_{n,k}(t)$$

$$\text{for } 0 < n < m;\ 0 \leqslant k \leqslant n.$$

The probabilities $P_{n,0}(t)$ are the wanted characteristics of the repair system in the context of cell survival. This modification of Model 1, biologically appealing as it is, meets with only limited success because a solution to the above system is not readily available.

Model 1.2. The basic assumption is that the explantation of cells blocks the repair process and the death of a cell is caused by any unrepaired or misrepaired lesion. In this case the function $F(N,t)$ is the product of the probability of the correct service, $(1-\beta)^N$, of all lesions and the probability, $P_0(t)$, that no unserved demands are left in the

system by time t, that is,

$$F(N,t) = \begin{cases} (1-\beta)^N P_0(t), & N > 0, \\ 1, & N = 0. \end{cases} \tag{15}$$

Using (6), (7), and (15), we obtain

$$F(N,t) = \begin{cases} (1-\beta)^N m\mu \int_0^t [1-e^{-\mu(t-u)}]^m P_{m+1}(u)\, du, & N > m, \\ [(1-\beta)(1-e^{-\mu t})]^N, & 0 \leqslant N \leqslant m. \end{cases} \tag{16}$$

Consequently, the overall survival probability is equal to

$$S(D;t) = \sum_{N=0}^{\infty} \frac{(\theta D)^N}{N!} e^{-\theta D} F(N,t), \tag{17}$$

with the function $F(N,t)$ given by (16).

In the event of immediate subculturing ($t = 0$), the dose–effect curve is expected to be exponential, that is,

$$S(D;0) = e^{-\theta D}, \tag{18}$$

which is intuitively appealing because the surviving cells are only those that bear no radiation-induced lesions. As to the limiting value of $S(D;t)$ as $t \to \infty$, it remains the same as in Case 1. If $N > m$, the function $F(N,t)$ can be represented as

$$F(N,t) = (1-\beta)^N m\mu \int_0^{\infty} \chi_{[0,t]}(u)[1-e^{-\mu(t-u)}]^m P_{m+1}(u)\, du,$$

where $\chi_{[0,t]}(u)$ is the characteristic function defined as

$$\chi_{[0,t]}(u) = \begin{cases} 1 & \text{if } u \in [0,t], \\ 0 & \text{otherwise.} \end{cases}$$

By virtue of the inequality

$$1 - e^{-\mu(t-u)} < 1$$

and the Lebesgue theorem on bounded convergence,

$$\lim_{t \to \infty} F(N,t) = (1-\beta)^N, \tag{19}$$

the same result being valid for $N < m$. It follows from (17) and (19) that

$$\lim_{t \to \infty} S(D;t) = e^{-\beta\theta D}.$$

2.2. *MODEL 2*

The repair system works without misrepair within the framework of this model (Figure 2). The radiation-induced lesions may be fixed spontaneously to form the flow of lesions fixed before entering the service system. Within the framework of this model such lesions are termed B lesions. The waiting time until the spontaneous fixation of a lesion is assumed to be exponential, and its mean value is denoted by $1/\nu$. The intensity of the flow of B lesions is proportional to the number of lesions waiting in the queue and hence decreases with time. We introduce the concept of A lesions in much the same way as for Model 1. Cell death is caused ultimately by any lesion of either type A or type B present at the moment of explantation. The longer the cell is kept in the medium without subculturing, the higher the probability for a lesion to be fixed spontaneously, but the probability of being served by the repair system increases concurrently. Thus, the assumptions of Model 2 also provide an explanation of the saturation effect. This model may be considered as a queueing system that is known as the *system with impatient customers* [28].

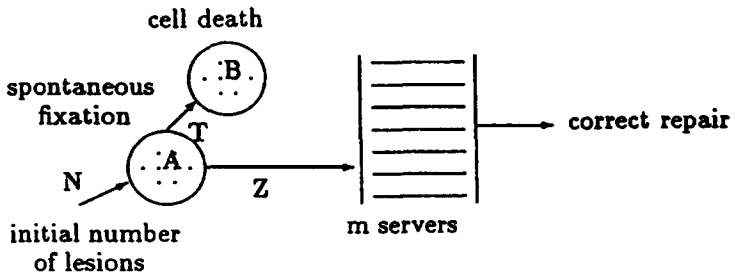

FIG. 2. Diagram of Model 2.

Let the initial number of lesions be equal to $N > m$. Then the states of the system are described by the joint probabilities, $P_k^0(t)$, of the following events: the flow of lesions of type B is empty, and there are exactly k lesions left in the system (being repaired or waiting for service) at the moment t. The probabilities $P_k^0(t)$ satisfy the following system of differential equations:

$$\frac{dP_0^0(t)}{dt} = \mu P_1^0(t),$$

$$\frac{dP_i^0(t)}{dt} = -i\mu P_i^0(t) + (i+1)\mu P_{i+1}^0(t) \qquad \text{for } 0 < i < m,$$

$$\frac{dP_{m+r}^0(t)}{dt} = -(\mu m + r\nu) P_{m+r}^0(t) + \mu m P_{m+r+1}^0(t)$$

$$\text{for } 0 \leqslant r \leqslant N-m,$$

$$\frac{dP_N^0(t)}{dt} = -[\mu m + (N-m)\nu] P_N^0(t).$$

This system can be integrated by the same procedure as the one applied to Equations (2). For $N > m$, the final solution is

$$P_k^0(t) = m\mu \binom{m}{k} \int_0^t [1 - e^{-\mu(t-u)}]^{m-k} e^{-k\mu(t-u)} P_{m+1}(u)\, du,$$

$$0 \leqslant k \leqslant m, \quad \text{(20a)}$$

$$P_{m+r}^0(t) = \frac{e^{-(\mu m + r\nu)t}}{(N-m-r)!} \left[\frac{m\mu}{\nu}(1 - e^{-\nu t})\right]^{N-m-r}, \qquad 0 \leqslant r \leqslant N-m,$$

(20b)

whereas for $N \leqslant m$ the solution is of the form

$$P_k^0(t) = \binom{N}{k} (1 - e^{-\mu t})^{N-k} e^{-k\mu t}, \qquad 0 \leqslant k \leqslant m. \qquad (21)$$

We consider the same two cases as in Section 2.1.

Model 2.1. Under this model, the lesions of type A are those waiting for service at the moment of subculturing, and the conditional survival

probability $F(N,t)$ is given by

$$F(N,t) = \begin{cases} \sum_{k=0}^{m} P_k^0(t) & \text{if } N > m, \\ 1 & \text{if } N \leqslant m. \end{cases} \tag{22}$$

It follows from (20) that

$$\sum_{k=0}^{m} P_k^0(t) = m\mu \int_0^t P_{m+1}^0(u)\, du$$

$$= \frac{1}{(N-m-1)!}\left(\frac{m\mu}{\nu}\right)^{N-m} B_{1-e^{-\nu t}}\left(N-m, 1+\frac{m\mu}{\nu}\right), \tag{23}$$

where $B_x(a,b)$ is the incomplete beta function.

To obtain the overall survival probability $S(D;t)$, we substitute (22) and (23) for $F(N,t)$ in formula (11). If irradiated cells are subcultured immediately after exposure, then $S(D;0)$ depends, in contrast to Model 1.1, on only the parameters θ and m:

$$S(D;0) = \sum_{N=0}^{m} \frac{(\theta D)^N}{N!} e^{-\theta D}.$$

Model 2.2. In this case, the set of lesions of type A includes those under service at the moment of subculturing. The conditional survival probability, given the initial number of radiation induced lesions N, is

$$F(N,t) = \begin{cases} P_0^0(t) & \text{if } N > m, \\ (1-e^{-\mu t})^N & \text{if } N \leqslant m. \end{cases} \tag{24}$$

The overall survival probability is given by (11) and (24), and the function $S(D;0)$ appears to be of the same form as for Model 1.2:

$$S(D;0) = e^{-\theta D}.$$

Since the series in (11) converges uniformly with respect to t, we may obtain the limiting survival probability as follows:

$$\lim_{t\to\infty} S(D;t) = \sum_{N=0}^{m} \frac{(\theta D)^N}{N!} e^{-\theta D} + \sum_{N=m+1}^{\infty} \frac{(\theta D)^N}{N!} e^{-\theta D}\left(\lim_{t\to\infty} F(t|N)\right). \tag{25}$$

For both versions of Model 2 we have

$$\lim_{t\to\infty} F(t|N) = m\mu\int_0^\infty P_{m+1}^0(u)\,du$$

$$= \left(\frac{m\mu}{\nu}\right)^{N-m-1}\frac{\nu}{(N-m-1)!}$$

$$\times\int_0^\infty e^{-(\mu m+\nu)u}(1-e^{-\nu u})^{N-m-1}\,du$$

$$= \left(\frac{m\mu}{\nu}\right)^{N-m-1}\frac{1}{(N-m-1)!}B\left(\frac{\mu m}{\nu}+1;N-m\right),$$

where $B(x;y)$ is the beta function. As one of the arguments of $B(x;y)$ is an integer, we can write

$$\lim_{t\to\infty} F(t|N) = \prod_{i=1}^{N-m}\frac{\mu m}{\mu m+i\nu},$$

which, in combination with (25), gives the limiting survival probability under the assumptions of Model 2.

2.3. *THE RELATIVE REPAIR CAPACITY*

A useful characteristic serving to compare different models is the relative capacity of a queueing system defined as the probability for *any* lesion, formed at the initial moment, to be repaired if the waiting time is allowed to be as long as necessary. For Model 1 the relative capacity is equal to the probability of correct repair, $1-\beta$. Under the assumptions of Model 2, all the lesions that have reached the repair system are ultimately repaired. Hence the relative capacity is given by the probability for a lesion to avoid spontaneous fixation before entering the repair system.

In terms of the theory of competing risks [29], the lifetime of a lesion can be defined as the waiting time until either the event of spontaneous fixation occurs (risk 1) or the lesion enters the repair system (risk 2). Let Z denote the moment when a lesion leaves the queue for being served and T the moment of spontaneous fixation. The variables Z and T being random, the lifetime of a lesion is thought of as the random variable $U=\min(T,Z)$. We assume that Z and T are independent random variables with cumulative distribution functions $G(t)$ and $H(t)$, respectively. The relative capacity, α, of the repair system described by Model 2 is the probability $\Pr(T>Z)$.

Given the initial number of lesions $N > m$, the conditional net probability that a lesion is still waiting for service at time t is

$$\begin{aligned} G(t|N) &= \Pr(Z > t|N) \\ &= \frac{1}{N} \sum_{i=1}^{N} \Pr(Z_i > t) \\ &= \frac{1}{N} \sum_{r=1}^{N-m} \Pr(Z_{m+r} > t), \end{aligned}$$

where Z_i is the random moment of the ith lesion entry into the repair system. It is clear that

$$\Pr(Z_{m+r} > t) = \sum_{j=N-r+1}^{N} P_j(t),$$

where $P_j(t)$ is the probability that by moment t there are exactly j lesions present in the system. The explicit expression for $P_j(t)$ is given by (3). Hence we have

$$\begin{aligned} G(t|N) &= \frac{1}{N} \sum_{r=1}^{N-m} \sum_{j=N-r+1}^{N} P_j(t) \\ &= \frac{1}{N} \sum_{j=1}^{N-m} jP_{j+m}(t). \end{aligned}$$

Since the competing risks under consideration are independent, the crude conditional probability $\alpha_N = \Pr(Z > T|N)$ can be represented as

$$\alpha_N = \int_0^\infty G(t|N)\, dH(t) = \frac{1}{N} \sum_{j=1}^{N-m} j \int_0^\infty P_{j+m}(t)\, dH(t),$$

where $H(t)$ stands for the net cumulative distribution function of T. As assumed initially, the distribution $H(t)$ is exponential with parameter ν. Therefore,

$$\begin{aligned} \int_0^\infty P_{j+m}(t)\, dH(t) &= \frac{\nu}{(N-m-j)!} \int_0^\infty (\mu m t)^{N-m-j} e^{-(\mu m+\nu)t}\, dt \\ &= \left(\frac{\mu m}{\mu m+\nu}\right)^{N-m-j} \left(\frac{\nu}{\mu m+\nu}\right). \end{aligned}$$

If we introduce the notation $\rho = \mu m/(\mu m + \nu)$, then

$$\alpha_N = \frac{1}{N} \sum_{j=1}^{N-m} j \rho^{N-m-j}(1-\rho).$$

The probability for a lesion to be left unrepaired is a function of irradiation dose. In view of the obvious fact that $\alpha_N = 0$ for $N \leqslant m$, we obtain

$$\begin{aligned}\alpha(D) &= \sum_{N=m+1}^{\infty} \alpha_N \frac{(\theta D)^N}{N!} e^{-\theta D} \\ &= (1-\rho) \sum_{N=m+1}^{\infty} \frac{1}{N} \left(\frac{(\theta D)^N}{N!} \right) e^{-\theta D} \sum_{j=1}^{N-m} j \rho^{N-m-j}.\end{aligned}$$

Thus the capacity of the system is given by $1 - \alpha(D)$.

It is worth noting that $\alpha(D)$ depends only upon the ratio $\nu/m\mu$, that is, the ratio of the two rates, associated with risk 2 and risk 1 or, to put it differently, of the intensities of the flows of lesions of type B and type A, respectively.

2.4. *MODEL 3*

From the above discussion it follows that both Model 1.2 and Model 2.2 display an exponential survival curve in the event of immediate subculturing, which typically is inconsistent with experimental data. This problem could be remedied by assuming an independent mechanism of superfast repair [30,31], but at the sacrifice of model simplicity, as this would increase the number of unknown parameters. That is why no consideration is given to these versions of models 1 and 2 in the application presented in the next section. For the same reason, a generalization given here refers to models 1.1 and 2.1 only.

The model under consideration allows both for misrepair, occurring with probability β, and for spontaneous lesion fixation with intensity ν. To avoid unnecessary repetitions we give the final expression for the probability of cell survival:

$$S(D;t) = \sum_{N=0}^{\infty} \frac{(\theta D)^N}{N!} e^{-\theta D} F(N,t), \qquad t > 0,$$

where

$$F(N,t) = \begin{cases} m\mu(1-\beta)^N \int_0^t P_{m+1}^0(u)\, du, & \text{for } N > m, \\ (1-\beta)^N & \text{for } 0 \leqslant N \leqslant m, \end{cases}$$

and P_{m+1}^0 is obtained from (20) at $r = 1$.

Being a combination of models 1.1 and 2.1, this model does not yield an exponential limiting dose–response relationship as $t \to +\infty$. Within the framework of Model 3,

$$\lim_{t \to \infty} S(D;t) = e^{-\theta D}\left[\sum_{N=0}^{m}(1-\beta)^N \frac{(\theta D)^N}{N!} + \sum_{N=m+1}^{\infty}(1-\beta)^N \frac{(\theta D)^N}{N!}\left(\prod_{i=1}^{N-m}\frac{\mu m}{\mu m + i\nu}\right)\right].$$

The relative capacity of the repair system can be derived from the following line of reasoning. Having entered the repair system, each lesion is repaired with probability $1-\beta$. Thus, the probability for any formed lesion to be repaired is given by the product of the capacities calculated for the two models:

$$1-\alpha(D) = (1-\beta)\left[1-(1-\rho)\sum_{N=m+1}^{\infty}\frac{1}{N}\left(\frac{(\theta D)^N}{N!}\right) \times e^{-\theta D}\sum_{j=1}^{N-m} j\rho^{N-m-j}\right].$$

This model is hierarchical in the sense that it can be reduced to the other two by eliminating the corresponding parameter ν or β.

3. DATA ANALYSIS

We apply the models introduced in Section 2 to the analysis of some published experimental data on recovery of cultured cells from radiation damage. In the following, use is made of only versions 1.1 and 2.1 of models 1 and 2. The reason for such a choice is given in Section 2.4. In the application described below, the model parameters were estimated by the minimum chi-squared method, and the statistical chi-square test was employed for the goodness-of-fit testing. The optimization procedure was based on the method by Nelder and Mead (see [32], pp. 451–454). As to Model 1.1, we proceeded from formulas (10) and (11) when applying this model to experimental data.

The first data set (Data Set 1) is taken from the paper of Little [33]. The experiments were performed on Chang liver cells (LICH) that were grown in the medium and allowed to reach a plateau phase of growth by two methods referred to as density inhibition and nutritional inhibition.

Example 1. Survival curves for density-inhibited stationary cultures irradiated with various doses of X-rays are presented in Figure 3. Either

the cells were subcultured to assay for colony-forming ability immediately after irradiation, or the cultures were returned to the incubator without change of medium and the cells were subcultured 6 or 12 h later. The repair of potentially lethal radiation damage seems to be essentially complete by 6 h.

Example 2. If under similar experimental conditions the culture medium was renewed immediately after irradiation, a different pattern was documented (Figure 4). A little repair occurred with delays as short as 6 h, although by 12–24 h it approached that in the cells kept in the conditioned medium.

Example 3. To determine whether potentially lethal damage repair was specifically associated with the density-inhibited state, similar experiments were performed with cultures whose growth was inhibited by medium exhaustion (nutritionally inhibited cultures). As can be seen from the survival curves depicted in Figure 5, the repair of radiation damage in nutritionally inhibited cultures continues up to 12 h and is similar to or greater in magnitude than that observed in the density-inhibited cultures. There is no evidence of saturation effect in this case.

Shown in Table 1 are the estimated values of the model parameters, all of them being biologically meaningful: m is the maximum number of

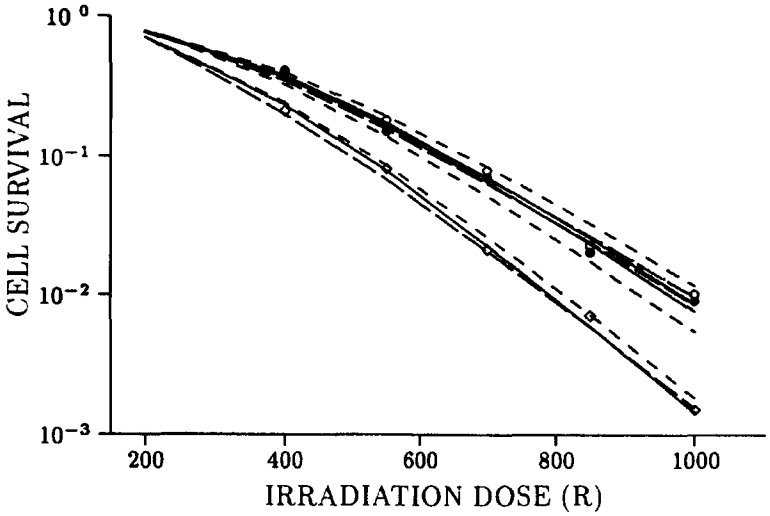

FIG. 3. Example 1. Experimental data: explanation immediately (◇), 6 h (●), and 12 h (○) after irradiation. Short dashes, Model 1, long dashes, Model 2; solid line, Model 3. See text for explanations.

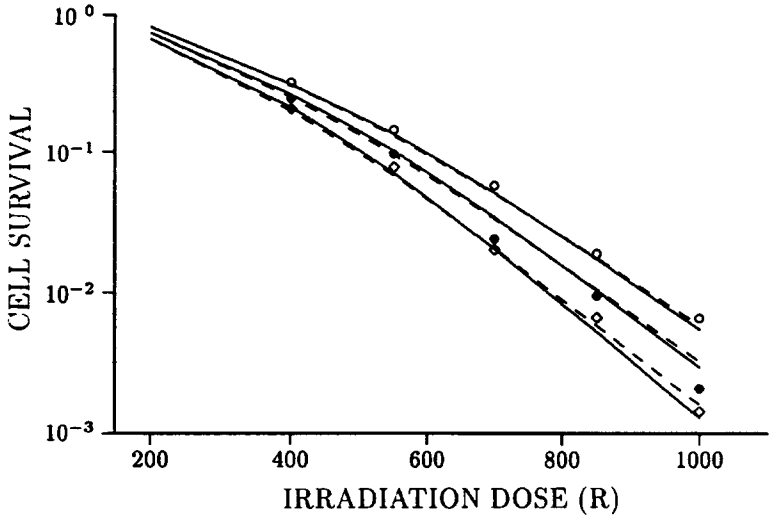

FIG. 4. Example 2. The same notation as in Figure 3. Models 1 and 3 yield identical dose–effect curves (solid lines).

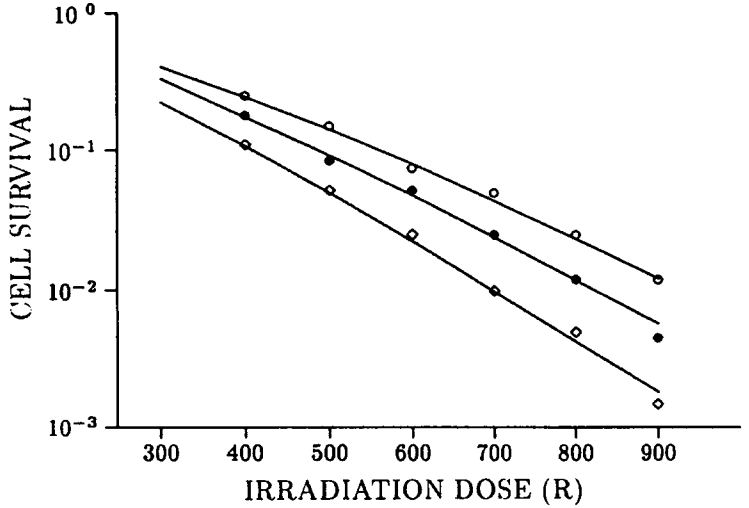

FIG. 5. Example 3. The same notation as in Figure 3. The theoretical curves produced by the three models are identical.

TABLE 1

Estimates of Parameters and χ^2 Values—Data Set 1

	m	θ	μ	β	ν	χ^2	d.f.
Example 1							
Model 1	3	1.20	3.63	0.08	—	3.45	11
Model 2	2	1.06	13.9	—	22.3	1.05	11
Model 3	3	1.24	7.00	0.06	9.80	0.90	10
Example 2							
Model 1	3	1.23	2.63	0.10	—	1.52	11
Model 2	2	1.07	2.65	—	0	1.65	11
Model 3	3	1.23	2.63	0.10	0	1.52	10

lesions that can be repaired at a time; θ is the mean number of primary lesions produced per unit (1 Gy = 100 rad) dose; μ is the service rate, which is the reciprocal of the mean duration (in hours) of the repair process; ν is the rate of spontaneous lesion fixation; and β is the misrepair probability.

There is close agreement between the estimates of the number of servers, m, provided by all three models when they are applied to the data of Examples 1 and 2. The same is true for the estimated values of the parameter θ. It is clear from Table 1 and Figures 3 and 4 that all the models provide a very good fit to the data, and none of them appears to be superior to the other two. As Example 2 suggests, this analysis does not call for the more complicated Model 3, which reduces to Model 1. The recovery of cells from potentially lethal damage is far from complete by $t = 12$ h under Model 1; this is corroborated by the limiting dose–effect curve computed for the same values of the model parameters. On the contrary, Model 2 predicts the completion of the repair processes by that time, providing a slightly better fit to experimental data in this specific case. As to Example 3, estimation of the parameters β and ν seems to be infeasible because of lack of information on the saturation effect. With the parameter values $m = 1$, $\theta = 0.95$, $\mu = 6.60$, $\beta = 0$, Model 1 provides a good fit ($p > 0.95$) in this case, and so does Model 2 at $\nu = 0$, all the other parameters being the same as for Model 1 (Figure 5). It is clear that the proposed models are unsuited for the analysis of truncated data like those presented in Example 3.

Another useful source of data (Data Set 2) is provided by the work of Weichselbaum and Little [34]. These data are summarized in the following two examples.

Examples 4. The plateau-phase cultures of human breast cancer cells MCF-7 were irradiated in different doses and subcultured either imme-

diately after irradiation or after a certain delay. The surviving fractions measured for various delay times after a single-dose irradiation are shown in Figure 6.

Example 5. Similar experiments were performed on cells of human melanoma C-145 in a wider dose range. The corresponding surviving fractions are presented in Figure 7.

As evident from Table 2 and Figures 6 and 7, the application of Model 3 is not warranted in both examples. Example 5 provides sufficiently strong evidence against Model 2, which is rejected at a significance level of less than 0.001.

Based on the parameter estimates displayed in Tables 1 and 2, the system capacity is typically greater under Model 2 than under Model 1. But it is Model 1 that demonstrates a good description of experimental data in all the examples considered above, though it does not consistently outperform the other two. The results in Tables 1 and 2 suggest that the radiosensitivity parameter θ is characteristic of the cell type (line), though its values may vary with the stage of cell culture growth [35], and the same is true for the β values. Since the μ values are

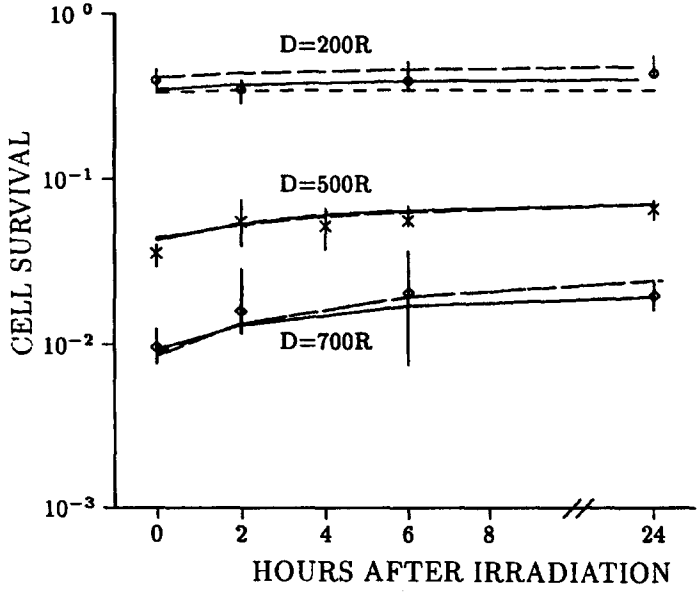

FIG. 6. Example 4. Open symbols correspond to experimental points with the variance shown by vertical bars. Cell cultures exposed to 200 R (○), 500 R (×), and 700 R (◇). Short dashes, Model 1; long dashes, Model 2; solid line, Model 3.

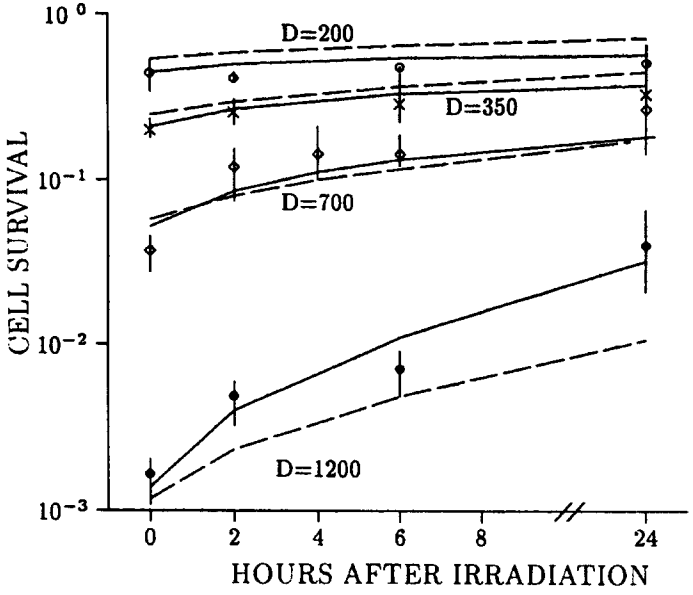

FIG. 7. Example 5. The same notation as in Figure 6. Experimental points: 200 R (○), 350 R (×), 700 R (◇), and 1200 R (●).

discrepant between Examples 1 and 2 (different culturing) and Examples 4 and 5 (different cell lines), they are likely to depend both on the type of cells and on the conditions of their culturing. Thus, the observed variations of the model parameters are intuitively appealing and biologically plausible. The estimated values of m suggest that the lesions subjected to repair processes are few in number, which is consistent

TABLE 2

Estimates of Parameters and χ^2 Values—Data Set 2

	m	θ	μ	β	ν	χ^2	d.f.
Example 4							
Model 1	3	1.31	17.9	0.48	—	2.08	9
Model 2	1	1.12	5.90	—	18.2	3.25	9
Model 3	1	1.03	15.8	0.40	16.4	1.04	8
Example 5							
Model 1	1	0.81	38.8	0.41	—	8.16	13
Model 2	1	0.87	11.1	—	7.70	39.4	13
Model 3	1	0.81	38.8	0.41	0	8.16	12

with small values of the critical number of hits resulting from the application of the multihit–one target model to cell survival after single-dose irradiation [22].

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