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Radiation Damage to a Dynamic Cell Population

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Abstract. Models for ionizing radiation damage to cells are surveyed. Examples are given for how radiation damage models can be combined with those standard cell population dynamics models which have, in the absence of radiation, a time development governed by a semi-group of non-negative operators. Such combined models are useful in analyzing observed radiation dose-rate effects: *direct* dose-rate effects when protracting a given dose leads to less damage, typically because of sublethal damage repair; or *inverse* dose-rate effects when protraction increases the amount of damage. Inverse dose-rate effects are usually due to cell cycle *redistribution*, i.e. to changes in the age-structure of a cycling cell population. Some recent results on combined models are surveyed: numerical methods; applications; and rather general analytic theorems on inverse dose-rate effects due to redistribution.

In the case of two acute doses separated by an adjustable time, effects caused by redistribution involve a tendency of the population age-structure for cells surviving the first dose to shift away from its immediate post-irradiation form, which itself is altered from the original due to the preferential elimination by the first dose of cells in more sensitive phases. Given time between the doses, what results is a generally restorative, though not neccessarily monotonic, "drift" of the age structure back towards the pre-irradiation form. The population resensitizes, compared to the population immediately following the first dose, which has an excess of resistant cells. Under suitable mathematical and biological restricitions, we correspondingly prove that for any mitosis rate, any dose-rate as a function of time, and any age-dependent radiation sensitivity function, an initially log-phase cell population is killed more effectively by any protracted dose, vs. an acute dose of the same magnitude. This concept of population resensitization is also applied to a model where cell transformation is correlated to cell killing and shows an inverse dose-rate effect. Resensitization driven by biological processes other than cycling (e.g. by reoxygenation) can also be incorporated into the models.

Key Words. Ionizing radiation; Liner-quadratic model; Cell cycle kinetics; Age-structure redistribution; Radiation resensitization.

1. Introduction. When a cell population is subjected to ionizing radiation, the resulting damage depends not only on the total dose but also on the time course of dose delivery, i.e. one has *dose-rate* effects. There are various reasons for the occurrence of dose-rate effects. One reason, of particular interest here, is that the cell population is dynamic. To model the situation one needs to combine models for population dynamics with models for radiation damage, much as in analyses of chemotherapy [Swan 1984; Cojocaru & Agur 1992; Webb 1996].

1.1. Population Dynamics. The population dynamics models we shall here consider are of a familiar type; all have, in the absence of radiation, a time development governed by a semi-group of eventually positive operators. An example is the linear, discrete-time formalism for a structured population governed by a non-negative, primitive $k \times k$ matrix M (i.e. all components of M are non-negative, $M \geq 0$, and there is a positive integer s such that all components of M^s are positive, $M^s > 0$). For example M could be an appropriate Leslie matrix [Tuljapurkar 1990] or Usher matrix. The time development is given by

$$\mathbf{p}_{n+1} = M\mathbf{p}_n, \ n=0,1,2,..., \quad \text{where } \mathbf{p}_0 \in R^k, \text{ and } \mathbf{p}_0 \geq 0. \tag{1.1.1}$$

In this example, there is a well defined asymptotic growth rate, determined by the Perron-Frobenius root r of M, and there is an asymptotically stable population structure $\mathbf{s}>0$, defined up to scale by $M\mathbf{s}=r\mathbf{s}$ [Minc 1988]. The ultimate size of the population is determined by $\omega(\mathbf{p}_0) \in (0,\infty)$, where $\omega > 0$ is the corresponding normalized eigen-form, i.e.

$$r^{-n}\mathbf{p}_n \underset{n\to\infty}{\rightarrow} \omega(\mathbf{p}_0)\ \mathbf{s} \equiv \sum_{i=1}^{k} \omega_i p_{0i}\ \mathbf{s}, \quad \text{where } \omega M = r\omega \text{ and } \omega(\mathbf{s})=1. \tag{1.1.2}$$

This result follows from the standard asymptotic estimate that $r^{-n}M^n \rightarrow \mathbf{s}\otimes\omega$, i.e. that $r^{-n}(M^n)_{ij} \rightarrow s_i \omega_j$ for all components.

Other relevant linear population dynamics models use a continuous time formalism, e.g. compartmental models [Yakovlev & Zorin 1988], or generalizations of the McKendrick/von-Foerster model (Sections 3 & 4), or diffusion-type models (Sections 4 & 5). Each of these models also has well defined asymptotic growth rate and asymptotically stable population structure. Non-linear models [Webb 1985; Tucker & Zimmerman 1988] are also important.

1.2. Preview. The radiation damage models which can be combined with such population dynamic models are presumably much less familiar here. In Section 2 we therefore discuss in detail a representative one, the incomplete repair LQ model. Section 3 briefly surveys combined models and the results that have been obtained using them. Sections 4 and 5 are devoted to some data and recent theorems on situations where prolonging a given dose results in more damage, in contrast to the situation, more typical for ionizing radiation, where prolongation results in sparing.

2. Ionizing Radiation. *2.1. Damage Mechanisms and Endpoints.* Ionizing radiation consists of high-energy particles whose tracks deposit energy in a cell [Goodhead 1987]. Dose, D, is measured in energy per unit mass. Cells are exquisitely sensitive to ionizing radiation; a dose of 1 Gray (≡ 1 Joule/kilogram) is not enough to raise the temperature of a cell 1/1000 of a degree, but it makes many thousands of ionizations in the cell's nucleus, of which a small minority, ≈40 in a human cell, quickly induce DNA double strand breaks. The DNA double strand breaks are repaired, misrepaired, or left as hopelessly unrepaired during the next half hour or so. At a dose of

1 Gray there often remain several misrepaired or hopelessly unrepaired DNA double strand breaks, enough to kill the cell at the next mitosis.

For sparsely ionizing radiation, such as x-ray or γ-ray photons, the kind of radiation usually used in tumor radiation therapy, there are many high energy particle tracks per Gray; the resulting DNA double-strand breaks are approximately Poisson distributed from cell to cell. For densely ionizing radiations, such as α particles or neutrons, which are of more concern in epidemiology and carcinogenesis, each track makes a cylinder of heavy damage and the number of tracks per Gray is small [Goodhead 1987].

There are many radiation damage endpoints. For brevity we shall here emphasize cell killing in the sense of clonogenic inactivation. Other cell-level endpoints and some molecular-level ones will also be discussed; we shall say very little about endpoints which are higher-level (e.g. concern organs, people, or human populations).

2.2. Dose and Dose-Rate. To describe the models for ionizing radiation damage in their simplest form, suppose, temporarily, that we have an unstructured population of N biologically identical cells, with $dN/dt=0$ in the absence of radiation (i.e., for the time being, no population dynamics). Situations where N must be regarded as a stochastic quantity are of some interest [e.g. Yakovlev & Yanev 1988; Tucker 1996]. But in the present analysis it will always be supposed that $N \gg 1$ and $N(t)$ will always be regarded as a deterministic function, not as a random one (on the other hand, certain other quantities, such as the number of misrepaired DNA double strand breaks per cell, are in principle regarded as stochastic, as described below).

We shall always suppose irradiation occurs within the finite time interval $[0,T]$. $D(t)$ denotes accumulated dose at time t. Thus $\dot{D}(t) \equiv dD/dt$ is radiation dose rate, $D \equiv D(T)$ is total dose and $\dot{D}/D(T)$ may be regarded as a probability density specifying the time course of dose delivery. In certain applications it is convenient to consider *acute* doses, corresponding to atoms in the cumulative distribution function $D(t)/D$, and then appropriate precautions are taken.

2.3. The LQ (Linear-Quadratic) Model. For cell killing (and for many other radiation damage endpoints), part of the damage can usefully be approximated by the familiar "linear" model, as follows. Suppose $dN/dt = -\alpha \dot{D} N$, where α is a constant (which depends on cell-type, radiation type, experimental conditions, etc.). This model is appropriate for radiation killing which is due to "1-track action" (e.g. does not involve interaction of DNA double strand breaks made by two different radiation tracks). The log surviving fraction assayed at large times is then:

$$\ln S \equiv \ln \left[N(\infty)/N(0) \right] = -\alpha D, \quad \text{where } D = D(T) = \int_0^T \dot{D} dt.$$

Thus for this "1-track" damage component, damage is linear in dose and the time course of dose delivery is irrelevant (no dose-rate effects).

However, a very distinctive feature of radiation damage is that often part of the damage: (a) is super-linear in dose; and (b) shows a *direct dose-rate effect,* i.e. decreases in severity the more a dose is spread out in time. The detailed properties of this additional damage component have long been controversial [Hanin et al. 1994]. Very many models, phenomenological and molecular, have been proposed. During the last decade, a strong consensus has emerged on the venerable LQ (linear-quadratic) model as the appropriate zeroth approximation. The LQ model comes in both phenomenological and molecular versions, but it is the phenomenological version which has the wider acceptance and is more useful for present purposes. Therefore we state this phenomenological version next, followed by a number of remarks about the history of the model, intuitive interpretations, molecular interpretations, mathematical reformulations, present status in the field of radiobiology, etc.

Phenomenologically, suppose radiation causes "sublethal lesions" which can be repaired or can combine pairwise to produce lethal lesions. Then one might guess that there is a damage component quadratic in dose which exhibits the direct dose-rate effect, as specified by the following equations.

$$\ln S = -\alpha D - \beta G D^2, \text{ where } \beta = constant. \tag{2.3.1}$$

Here $G_{acute}=1$, but for prolonged irradiation $G<1$ due to repair. Specifically we shall here always take G as the generalized Lea-Catcheside function G_λ [Lea 1955; Brenner et al. 1991]

$$G = G_\lambda = 2\int_0^T f(t)dt \int_0^t e^{-\lambda(t-t')} f(t')dt', \text{ where } f(t)=\dot{D}(t)/D. \tag{2.3.2}$$

Here $\lambda>0$ is a constant repair rate, and, typically, 1 min $\leq 1/\lambda \leq$ several hours. Some comments on these equations are needed.

(2.3.3) History. The LQ model, in approximately the above form, is more than a half-century old; many of the early experimental and theoretical results are due to the great English radiobiologist Douglas Lea and his coworkers. The particular version that uses Eq. (2.3.2) is essentially the *incomplete repair LQ model* [Thames & Hendry 1987]. The (incomplete repair) LQ model is now heavily used, worldwide, in radiobiology, radiotherapy planning, cancer epidemiology, investigation of radioactive accidents, etc. Some of the main contributions to its present formulation and applications are surveyed and referenced by Barendsen [1982], Dale [1985], Thames and Hendry [1987], Fowler [1989], Tucker and Travis [1991], and Brenner et al. [1991; 1995].

(2.3.4) Interpretation. Eq. (2.3.2) can be interpreted intuitively by saying that near time t' some sublethal lesions are added by radiation, the average per-cell number being proportional to $\dot{D}(t')dt'$; these sublethal lesions may be repaired at rate λ during a time interval $t-t'$, but those that remain at time t can interact pairwise with sublethal lesions made near time t, with average per-cell number $\propto \dot{D}(t)dt$. Thus the model (like many other models of radiation damage) ascribes the direct dose-rate effect to repair which can occur in the time between arrival of two radiation tracks. One main alternative to such models involves the possibility that repair mechanisms could saturate if dose is delivered very rapidly [survey and references in Hanin et al. 1994]; such saturation can lead to a direct dose-rate effect even if pairwise misrepair of sublethal lesions is not significant.

(2.3.5) Acute Irradiation. In applications of (2.3.2), including some variational calculations [Brenner et al. 1994], it is convenient to consider acute doses. Then one must be careful because G_λ is a bilinear functional of f, and we shall here always mean the appropriate limit of a very large constant f during a very short time. The formalism seems to cry out for replacing f by the corresponding measure [compare Diekmann et al. 1994], but this has not to our knowledge been

carried through systematically.

(2.3.6) Molecular Observations and Models. Experimentally, one often finds molecular damage due to pairwise misrepair of repairable, radiation induced DNA double strand breaks. For example, this is seen in chromosome aberrations assayed using fluorescent in situ hybridization [overviews and references in Simpson and Savage 1995 or A. Chen et al. 1995]. Mathematical models of such processes involve stochastic mass-action chemical kinetics [overviews and references in Sachs et al. 1992]. One analyzes the time development of integer-valued random variables, e.g. the per-cell number of "sublethal lesions" (presumably, unprocessed DNA double strand breaks) and the number of lethal misrepairs (putatively, certain illegitimate DNA recombination events).

(2.3.7) Kinetic Reformulation. Eq. (2.3.2) can be rewritten in differential equation form. One method is to note that, for the case of sparsely ionizing radiation, unprocessed DNA double strand breaks and hopelessly misrepaired ones remain very nearly Poisson distributed from cell to cell during the entire repair-misrepair process [Sachs et al. 1992]. In this Poisson distributed case, one can average the stochastic mass-action equations (discussed above) to get ODEs. Specifically, let $u(t)$ denote the average per-cell number of unprocessed DNA double strand breaks, with $u(0)=0$, and let $N(t)$ denote the number of cells which have not yet suffered an irremediably fatal pairwise misrepair event. Then [Albright 1989, Sachs et al. 1992] there are constant positive rates c, λ, κ such that

$$(A)\quad \dot{u}=c\dot{D}-\lambda u-2\kappa u^2, \qquad (B)\quad \dot{N}=-\tfrac{1}{2}\kappa u^2 N. \tag{2.3.8}$$

Eq. (2.3.8A) has often been motivated from ordinary chemical kinetics, without reference to stochastic processes [e.g. Curtis 1986]. The following connection between Eqs. (2.3.8) and (2.3.2) is well known [e.g. Thames and Hendry 1987].

(2.3.9) Proposition. Suppose the term $-2\kappa u^2$ in (2.3.8A) is negligible, and regard the surviving fraction S as $S=N(\infty)/N(0)$. Then (2.3.8) implies $\ln S=-\beta G D^2$, where $\beta=(\kappa c^2/4\lambda)$ and G is given by (2.3.2).

Proof. Neglecting the indicated term and integrating (2.3.8A) gives

$$u(t) = c\int_0^t \dot{D}(t')e^{-\lambda(t-t')}dt'.$$

Inserting into (2.3.8B), integrating, and rearranging integrals to derive the identity

$$\int_0^\infty dt\left[\int_0^t \dot{D}(t')e^{-\lambda(t-t')}dt'\right]^2=\frac{1}{2\lambda}G_\lambda \tag{2.3.10}$$

gives $\ln S=\beta G D^2$ •

In practice, the error made by neglecting $-2\kappa u^2$ in (2.3.8A) is often very small [Thames and Hendry 1987], and then either (2.3.2) or (2.3.8) is used, as convenient.

(2.3.11) Current Status of the LQ Model. The LQ model is an intentional oversimplification of a complex situation. Provided they are not taken too literally, such intentional oversimplifications can have profound utility and unifying power. For example, for many years the oversimplification that a solid is an assembly of harmonic oscillators has greatly clarified much of solid state physics,

and the oversimplification of a spatially homogeneous and isotropic universe has been the basic starting point for cosmology. At worst, the LQ model summarizes, in approximate but quantitative form, a truly enormous body of radiobiological data and clinical experience. We shall use it throughout, as a representative radiation damage model.

3. Combined Models. *3.1. Examples.* Combining models of population dynamics with models of radiation damage typically involves considering effects during short time intervals and using differential or difference equations (rather than, e.g., Volterra integral equations [compare Webb 1985; Diekmann et al. 1994]). The method can be illustrated by assuming a cell population structured according to age, a, whose time evolution in the absence of radiation is governed by the McKendrick/von-Foerster equations [Webb 1985]. If the quadratic killing term, corresponding to $\beta G D^2$ in Eq. (2.3.1) is negligible, then the appropriate combined model is

$$(A)\ \partial n/\partial t + \partial n/\partial a = -\gamma n - \alpha \dot{D}(t) n\ ,\ \ (B)\ n(0,t) = 2\int_0^\infty \gamma n(a,t) da, \qquad (3.1.1)$$

with $n(a,t)$ the density, and $\gamma(a)$, $\alpha(a)$ age-dependent mitosis rate and radiation sensitivity respectively.

This approach can be generalized. For example, suppose we wish to take into account the following additional biological effects and results: (a) the quadratic killing term in Eq. (2.3.1); (b) the fact that, even in the absence of radiation, the most variable part of the cell cycle is G1; (c) radiatively produced sublethal damage which causes perturbations of the vital parameters, such as cell cycle delays at the G1/S border and during M, or homeostatic mechanisms, or apoptosis [Meyn et al. 1994]; (d) a natural apoptosis rate. Then we may introduce the average number of sublethal lesions, u, as an additional structure variable, and introduce two densities $n_i(a,u,t)$ $(i=1,2)$ for the G1 phase and the remaining phases (S+G2+M) respectively, subject to the following equations.

$$\partial n_i/\partial t + \partial(v_i n_i)/\partial a + \partial\left[(c_i \dot{D} - \lambda_i u - 2\kappa_i u^2) n_i\right]/\partial u = -(\gamma_i + \alpha_i \dot{D} + \tfrac{1}{2}\kappa_i u^2) n_i . \qquad (3.1.3)$$

$$n_2(0,u,t) = \int du'\, da'\, K_1(u,u',a') n_1(a',u',t), \qquad (3.1.4)$$

$$n_1(0,u,t) = \int du'\, da'\, K_2(u,u',a') n_2(a',u',t). \qquad (3.1.5)$$

Here the various coefficients depend on a; v_i and γ_i can also depend on u; the time development (2.3.8A) is incorporated via the characteristic equations [Tucker & Zimmerman 1988]. Eq. (3.1.4) describes cells "graduating" from G1 to the start of S and Eq. (3.1.5) describes mitosis. The use of two subpopulations in Eqs. (3.1.3)-(3.1.5) is analogous to the approach sometimes used to analyze how grandchildren's demographics are influenced by those of their grandparents [K. Wachter, private communication].

3.2. Results: Overview. A number of results have been obtained using such combined models. The most straightforward approach, and probably in the long run the most powerful, is computer-based. One carries out Monte-Carlo simulations for the time development of an age-structured cell population, or uses numerical approximations to equations like those in Subsection 3.1 [Yakovlev & Zorin 1988; King et al. 1988; Dillehay 1990; Zaider & Wuu 1995]. As is very typical, a numerical approach is highly flexible, and allows incorporation of a number of radiobiological effects, including those discussed in the previous Subsection. However, as is also typical, the number of adjustable parameters in such an approach is usually very large, as indicated, e.g., by the plethora of adjustable functions in (3.1.3-5). At present, the data, though very extensive, are not of sufficiently high precision to really determine more than a few parameters.

It is thus important to supplement numerical approaches with analytic theorems, valid for <u>all</u> relevant choices of the adjustable parameters or functions. General theorems provide guides and overviews. We next give samples of such comprehensive results.

4. Resensitization Models. *4.1. Biology.* One application of the combined models is that they can be used to quantify the phenomenon of *resensitization* [Hlatky et al. 1994], which in some circumstances tends to make protracted irradiation of a diverse cell population kill more effectively than an acute dose. Elkind and coworkers [e.g. Elkind et al. 1965] originally gave experimental results for a particular kind of resensitization, due to cell-cycle redistribution. The intuitive picture was, roughly, as follows. Acute irradiation of a cycling cell population preferentially kills cells in radiosensitive age brackets and spares cells in radioresistant age brackets. Assuming sublethal damage is negligible, the average radiosensitivity of the cell population is consequently decreased, and a second radiation dose immediately after the first is less effective. But, with time, the cycling alters the population age-structure and on balance tends to restore a higher average radiosensitivity, i.e the population gradually resensitizes due to cell cycle redistribution. Generalizing to an arbitrary time-pattern of dose delivery, one may find *inverse dose-rate effects*, i.e. more killing when a radiation interval is prolonged.

A number of subsequent experiments verified the phenomenon, though it often tends to be masked by the classic (direct) dose-rate effect attributable to sublethal damage repair (Subsection 2.3). Elkind's picture was subsequently generalized [Thames & Hendry 1987; Hlatky et al. 1994] to include other types of cellular diversity and include resensitization induced by biological processes other than cycling (such as reoxygenation).

4.2. Theorems on Resensitization. Recently, some analytic theorems on resensitization have been proved [Hlatky et al. 1994; P-L Chen et al. 1995; Hahnfeldt & Hlatky 1995]. The details of the theorems can get quite lengthy, but the most essential features of both the phenomenon and the proofs can be illustrated with a simple example. Use the linear, discrete-time, discrete-structure-space model of Subsection 1.1 and assume the LQ model with negligible quadratic term. Then we may introduce a $k \times k$ matrix A for age-dependent radiation sensitivity, with positive diagonal entries α_{ii} and zero off-diagonal terms, such that an acute radiation dose D_n delivered just after the n^{th} time step carries the population vector $\mathbf{p}_n$ into $\hat{\mathbf{p}}_n = [\exp(-D_n A)]\mathbf{p}_n$, where the matrix exponential is used. Now suppose that $\mathbf{p}_0 = \mathbf{s}$, the asymptotically stable age distribution, and compare an acute dose D given after the 0^{th} step with 2 doses $D/2$, one given after the 0^{th} step and one given after the n^{th}. In any case the ultimate size of the population is determined by $\omega(\hat{\mathbf{p}}_n)$, where ω is again the eigen-form (1.1.2) to Perron-Frobenius root r. Thus we must compare the two quantities of the following

$$\textit{Theorem:} \qquad \omega\left[e^{-DA/2} M^n e^{-DA/2} \mathbf{p}_0\right] \le \omega\left[M^n e^{-DA} \mathbf{p}_0\right]. \tag{4.2.1}$$

Proof. By convexity of the exponential function

$$\exp[-½(\alpha_{jj}+\alpha_{ii})D] \le ½\left[\exp(-\alpha_{ii}D)+\exp(-\alpha_{jj}D)\right]. \tag{4.2.2}$$

Consequently: $\omega(e^{-\frac{1}{2}DA}M^n e^{-\frac{1}{2}DA}\mathbf{s}) \equiv \Sigma\omega_i e^{-\frac{1}{2}\alpha_{ii}D}(M^n)_{ij} e^{-\frac{1}{2}\alpha_{jj}D} s_j$

$$\leq \frac{1}{2}\left[\omega(e^{-DA}M^n\mathbf{s}) + \omega(M^n e^{-DA}\mathbf{s})\right] = \frac{1}{2}\times 2r^n\omega(e^{-DA}\mathbf{s}) = \omega(M^n e^{-DA}\mathbf{s}) \bullet$$

The inequality is strict unless there is no fluctuation at all in radiosensitivity, i.e. unless $A \propto$ *Identity Matrix*. Assuming henceforth that A is not $\propto$ *Identity Matrix*, the two separated doses will always lead to more killing than a single combined dose, regardless of details on age-dependent radiosensitivity. This general inequality is the essential feature of the inverse dose-rate effect caused by resensitization through cell-cycle redistribution.

(4.2.3) Remarks. (a) The assumption, $\mathbf{p}_0=\mathbf{s}$, that prior to irradiation the cells were "exponentially cycling", i.e. had the stable age-pattern, is essential to the result. (b) Eq. (4.2.2) can be interpreted in terms of "overkill" (aka "saturation"), roughly, that the dose wasted in trying to kill the same cell twice is less if sensitive and resistant subpopulations are exchanged half-way through dose administration. This interpretation catches an essential feature of resensitization; for example if D is sufficiently small that the matrix $\exp[-DA]$ can be replaced by $1-DA$, then there is no overkill and there is also no resensitization, since the inequality (4.2.1) then becomes an equality. (c) Resensitiztion need not be monotonic; i.e. increasing n above need not always mean decreasing ultimate size, although the case $n=0$ always gives the largest ultimate size. (d) Examining the proof shows that the timing of the acute dose of magnitude D is not relevant; one has the same ultimate amplitude whether it is administered after the 0^{th} step or after any other step.

4.3 Some Generalizations. Theorem (4.2.1) can be generalized in a number of ways. The fact that we had two equal dose fractions is irrelevant; the same result holds for any number of fractions with any apportionment of dose. For example, if $a+b+c=1$, then by essentially the same argument as above,

$$\omega(e^{-aDA}M^{n-i}e^{-bDA}M^i e^{-cDA}\mathbf{s}) \leq \omega(M^n e^{-DA}\mathbf{s}).$$

Thus, for the population model (1.1) and 1-track killing, survival for an acute dose is always larger than for any split dose pattern, again regardless of how radiation sensitivity varies with age.

Moreover, the only essential features of the population model used were linearity, time-translation invariance when there is no radiation, existence of an asymptotic growth rate, and existence of an asymptotically stable population structure. For example, consider a continuous time population dynamics model which has these properties and suppose there is a continuous structure variable u (or several, collectively designated u). Then Theorem (4.2.1) and its proof can be generalized, for an arbitrary function $\alpha(u)$, allowing any sequence of acute doses and/or continuous dose rates. Conceptually, all one needs to do is replace everywhere the action $M^i\mathbf{p}$ by the action

$$\int du' P(u,u';t-t')n(u',t'), \text{ where } t-t' \Leftrightarrow i$$

and P is the appropriate semi-group element (i.e., the Green's function or propagator). This generalization is far reaching; for example it allows (linear) models in which one has a subpopulation of quiescent cells [Gyllenberg & Webb 1990] and allows diverse cell populations composed of subpopulations. Of course technicalities arise in the case of continuous structure variables due to the fact that the space of possible population functions is infinite dimensional. But for the

McKendrick/von-Foerster formalism 3.1.1 the appropriate generalization of theorem 4.2.1 has been carried out in detail [P-L Chen et al. 1995; Hahnfeldt & Hlatky 1995].

5. Comparing Direct and Inverse Dose-Rate Effects. *5.1. Sublethal Damage Repair vs. Resensitization.* Because the quadratic, βGD^2, cell killing term of Subsection 2.3 was neglected in the models of the preceding Section, these models do not adequately exhibit the competing effects, found experimentally, of sublethal damage repair and resensitization. We now give a model [Hlatky et al. 1994] which has been used to quantify both effects simultaneously and has sufficiently few adjustable parameters to make some numerical estimates possible in specific experiments. The model is also more general than the one given in Section 4 for the folowing reasons: it applies when all or part of the radiobiological diversity is due to differences other than differences in cell age (e.g. is due to microenvironmental, epigenetic or genetic differences); and it also applies if some resensitization is due to biological changes not directly determined by changing cell-cycle phase. The model will be applied first to cell killing and then to another cell-level endpoint. It is designated the LQR model because it incorporates some resensitization effects into the LQ model, and can be used [Brenner et al. 1995] as a unified approximation for all of Withers' famous "4-R's of radiotherapy": repair, repopulation, redistribution and reoxygenation [e.g. Withers 1992]. However the model is limited to cases where the variance in radiosensitivity and the dose delivered are both "small", in the sense made precise by Eq. (5.2.5) below.

5.2. The LQR Model. The model uses a structured cell population with the structure variable being radiosensitivity α itself; denote the density by $n(\alpha,t)$. In the absence of irradiation, the density n is taken to follow an Ornstein-Uhlenbeck diffusion process, which tends to create a Gaussian population structure [Bhattacharaya & Waymire 1990]. It will be useful to denote by $g(\alpha;\bar{\alpha})$ the Gaussian distribution having variance σ^2 and mean $\bar{\alpha}$, and denote by $h(\alpha,t)$ the value of g when σ is fixed but $\bar{\alpha}$ is a time-dependent function $\bar{\alpha}(t)$, i.e.

$$g(\alpha;\bar{\alpha}) = \frac{1}{\sigma\sqrt{2\pi}}\exp\left[-(\alpha-\bar{\alpha})^2/2\sigma^2\right], \quad h(\alpha,t)=g(\alpha,\bar{\alpha}(t)). \tag{5.2.1}$$

Let L be the Ornstein-Uhlenbeck diffusion operator with parameters ν, α_0 and σ^2, i.e.

$$Ln \equiv (\partial/\partial t)n(t,\alpha) - \nu(\partial/\partial\alpha)[(\alpha-\alpha_0)n+\sigma^2(\partial n/\partial\alpha)]. \tag{5.2.2}$$

In the absence of irradiation we assume the Ornstein-Uhlenbeck PDE Ln=0, which implies that total number of cells, $N=\int_{-\infty}^{\infty} n d\alpha$, is constant in the absence of irradiation; thus proliferation is here being neglected (though, by a minor extension [Hlatky et al. 1994], it can be included without complicating the following proofs significantly). Ln=0 also implies that there is an asymptotically stable, time-independent population structure, proportional to a Gaussian with mean α_0 and variance σ^2, i.e., for t sufficiently large and no irradiation, $n \rightarrow Ng(\alpha;\alpha_0)$. In Eq. (5.2.2) ν is a resensitization rate; roughly speaking, this means that if the average population radiosensitivity at one instant deviates from the value, α_0, for the asymptotically stable population structure $g(\alpha;\bar{\alpha})$ there is a tendency (modulated by diffusion effects) to return to this average value at rate ν. The idea is that detailed models, such as that of the preceding Section, do show an overall tendency to restore the average sensitivity after a perturbation, so in a general population which has many kinds of diversity it may be useful to approximate this restoring tendency by simple exponential decay.

Now suppose the population is irradiated with dose-rate $\dot{D}$, where *Supp* $\dot{D} \subseteq [0,T]$. Combining with the LQ radiation damage model by the method of Section 3, and assuming that prior to irradiation the population structure is the asymptotically stable one, one has as the LQR model's underlying equations

$$(A)\ du/dt = c\dot{D} - \lambda u - 2\kappa u^2, \quad (B)\ Ln = -F(\alpha,t)n, \quad (C)\ n(\alpha,0) = N(0)g(\alpha;\bar{\alpha}); \tag{5.2.3}$$

$$\text{where}\quad F(\alpha,t) = \alpha\dot{D}(t) + (1/2)\kappa u^2(t). \tag{5.2.4}$$

If $\dot{D}$ describes a single acute dose, the LQR model is equivalent to a special case of the Gaussian diversity model of Schultheiss et al. [1987]. Formally, one allows negative values of α in such Gaussian models, but the negative values are not realistic, so the whole formalism applies only when $n(\alpha,t)$ is negligibly small for α negative and any t, which is a self-consistent assumption in many specific applications [Hlatky et al. 1994]. Sufficient conditions for self-consistency are the small-variance/small-dose restrictions

$$\alpha_0^2 \gg \sigma^2, \quad \alpha_0 \gg \sigma^2 D. \tag{5.2.5}$$

In general, non-Gaussian distributions of α would have to be introduced, much as in corresponding situations for quantitative genetics [Karlin 1987]. However, when the restrictions (5.2.5) hold, even non-Gaussian models can be approximated using Eq. (5.2.9) below [Brenner et al. 1995].

While the implications of the LQR model were originally obtained by integrating the PDE (5.2.3B) [Hlatky et al. 1994], it subsequently turned out [Brenner et al. 1995] that the initial condition (5.2.3C) enables one to replace the PDE (5.2.3B) by ODEs, as shown by the following results. We start with some formal properties of L, obtained by direct computation, which will be useful several times. Let $h(\alpha,t)$ be as in (5.2.1). Suppose $\bar{\alpha}(t)$, $a(t)$, and $H(\alpha,t)$ are smooth functions.

(5.2.6) *Lemma.* $(A)\ Lh = (h/\sigma^2)\left[(d\bar{\alpha}/dt) + \nu(\alpha-\alpha_0)\right](\alpha-\bar{\alpha});$

$(B)\ L(aH) = (da/dt)H + aLH; \quad (C)\ L(\alpha h) = \alpha Lh + (\alpha + \alpha_0 - 2\bar{\alpha})h.$

(5.2.7) Lemma. Eqs. (5.2.3BC) and (5.2.4) imply that for $t \geq 0$, $n(\alpha,t) = a(t)g(\alpha;\bar{\alpha}(t))$, where g is given by (5.2.1), $\bar{\alpha}(0) = \alpha_0$, $a(0) = N(0)$, and

$$d\bar{\alpha}/dt = -\nu(\bar{\alpha}-\alpha_0) - \sigma^2\dot{D}(t), \quad da/dt = [-\bar{\alpha}(t)\dot{D}(t) - (1/2)\kappa u^2]a. \tag{5.2.8}$$

Proof. Substituting $n = ah$ into (5.2.3B) and using (5.2.6AB), together with (5.2.8) gives an identity. The Lemma thus follows by uniqueness of solutions to the initial value problem • Of course (5.2.8) can be integrated. The case of main interest is, as discussed above, when the term $2\kappa u^2$ in (5.2.3A) is negligible. Assuming that case, and again identifying S with $N(\infty)/N(0)$, integration gives the following

(5.2.9) *Theorem.* $\ln S = -\alpha_0 D - \beta G_\lambda D^2 + ½\sigma^2 G_\nu D^2.$

Here β and G are given by (2.3.9) and (2.3.2). *Proof.* Integrate (5.2.8) and use (2.3.10) •

Generally speaking, G decreases when a given dose is protracted. For example suppose D is fixed and $\dot{D}$ is the constant D/T, with irradiation time T adjustable. Then regarding $G = G_\lambda$ as a function of T we have

$$G(T) = 2(\lambda T)^{-2}\left[\lambda T - 1 - e^{-\lambda T}\right], \quad \frac{dG}{dT} < 0, \quad G \underset{T\to 0}{\rightarrow} 1, \quad G \underset{T\to\infty}{\rightarrow} 2/\lambda T, \tag{5.2.10}$$

and similarly for G_ν. With G_λ, G_ν decreasing functions of T, the opposite signs in Theorem (5.2.9) show very directly both the direct and inverse dose-rate effects. To disentangle the effects [Brenner et al. 1995], one must rely on the fact that typically ν^{-1} is of the order of a cell-cycle time, and thus considerably larger than λ^{-1} (Subsection 2.3 above).

5.3 Transformation Models. Experimentally, the most convincing examples of inverse dose rate effects are not for cell killing but for cell transformation (roughly: an *in vitro* surrogate for carcinogenesis). the examples usually occur for densely ionizing irradiation, where the quadratic sublethal damage repair term is so small the resensitization effects are not masked. Possible inverse dose-rate effects for carcinogenesis by densely ionizing radiation are of some concern [Brenner 1994b, Elkind 1994b]. Biological models of inverse dose-rate effects in this case [Rossi and Kellerer 1985; Brenner & Hall 1990; Elkind 1991; Brenner et al. 1993; Elkind 1994a] invoke resensitization due to cell cycle redistribution as the underlying phenomenon. We give here a quantification of such models.

The notation of Subsection 5.2 will be used throughout the following. Suppose there is a cell population modeled by $n(\alpha,t)$ obeying Eqs. (5.2.3) and (5.2.4). Suppose in addition there is a transformed cell population, modeled by $m(\alpha,t)$, having the same intrinsic kinetics and radiosensitivity as the untransformed population, and made by transforming some of the untransformed cells at a rate proportional to cell killing, i.e.

$$Lm = -Fm + \varepsilon F n; \quad m(\alpha,0) = 0, \tag{5.3.1}$$

Here $\varepsilon \ll 1$ is a parameter, assumed constant everywhere in structure-space, giving the ratio (transformation)/(killing + transformation). The most essential feature of this model is an assumed correlation between killing and transformation [Elkind 1994a], which is plausible on a molecular level since both are presumably due to misrepaired or unrepaired DNA double strand breaks.

(5.3.2) *Lemma.* $(m/\varepsilon) = \alpha b(t)n + c(t)n$, where $\dot{b} + \nu b = \dot{D}$, $\dot{c} = \nu b(2\overline{\alpha} - \alpha_0) + ½\kappa u^2$.

Proof. Use (5.2.6ABC) and proceed as in the proof of Lemma (5.2.7) •

An empirically observed quantity is the fraction, at large times, of surviving cells which are transformed. (5.3.2) gives for this quantity

$$\text{(5.3.3)} \quad \textit{Theorem}: \ \frac{1}{N(\infty)}\int_{-\infty}^{\infty} m(\alpha,\infty)\,d\alpha = \varepsilon[\alpha_0 D + \beta G_\lambda D^2 - \sigma^2 G_\nu D^2].$$

Proof. Integrate Eq. (5.3.2) and proceed as in the proof of Theorem (5.2.9) •

Remarks. (a) The negative term in Eq. (5.3.3) corresponds to the inverse dose-rate effect. The theorem, together with explicit examples such as (5.2.10), again show very explicitly the competing effects of the direct and inverse dose-rate effects, due to sublethal damage repair and resensitization respectively. (b) The missing factor ½ in (5.3.3) compared to (5.2.9) is not a misprint. (c) The fact that formally (5.3.3) could be negative for $\beta G_\lambda < \sigma^2 G_\nu$ and large D is related to the Gaussian idealization discussed above (compare Eq. 5.2.5).

6. Discussion. Qualitative models of radiation damage to dynamic cell populations, stated biologically and analyzed via detailed experiments, have been very heavily pursued for a long time. Quantitative modeling involving anything more than simple algebra still, for the most part, involves a disagreeable choice between oversimplification and overparametrization. This caveat applies to our example of the competition between sublethal damage repair and resensitization as well as to many other effects. However, quantitative models are essential for the understanding the underlying phenomena. The relevant mathematics is very rich and interesting, as are the experimental and clinical results. Confronting the mathematics with the observations is a formidable, often discouraging, challenge but the effort has helped uncover essential mechanisms of practical importance, and will continue to do so.

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