PNL-SA--18754

DE91 004737

PHENOMENOLOGICAL MODELS

L. A. Braby

September 1990

Prepared for
DOE Conference on
Molecular and Biological
Effects of Radiation,
Woods Hole, MA
September 4-7, 1990

Work supported by the Office of
Health and Environmental Research
(OHER), U.S. Department of Energy
under Contract DE-AC06-76RLO 1830

Pacific Northwest Laboratory
Richland, Washington 99352

MASTER**DISCLAIMER**

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

PHENOMENOLOGICAL MODELS

L. A. Braby
Pacific Northwest Laboratory
Richland, WA 99352

ABSTRACT

The biological effects of ionizing radiation exposure are the result of a complex sequence of physical, chemical, biochemical, and physiological interactions which are modified by characteristics of the radiation, the timing of its administration, the chemical and physical environment, and the nature of the biological system. However, it is generally agreed that the health effects in animals originate with changes in individual cells, or possibly small groups of cells, and that these cellular changes are initiated by the ionizations and excitations produced by the passage of charged particles through the cells. One way to begin a search for an understanding of health effects of radiation is through the development of phenomenological models of the response. Many models have been presented and tested in the slowly evolving process of characterizing cellular response. Different phenomena (LET dependence, dose rate effect, oxygen effect etc.) and different endpoints (cell survival, aberration formation, transformation, etc.) have been observed, and no single model has been developed to cover all of them. Instead, a range of models covering different endpoints and phenomena have developed in parallel. Many of these models employ similar assumptions about some underlying processes while differing about the nature of others. An attempt is made to organize many of the models into groups with similar features and to compare the consequences of those features with the actual experimental observations. It is assumed that by showing that some

assumptions are inconsistent with experimental observations, the job of devising and testing mechanistic models can be simplified.

INTRODUCTION

Models, ranging from vague conceptual descriptions to detailed mathematical expressions, play a major part in the development of most sciences. Models serve many different purposes, and take on different properties in accordance with those purposes. When previously unknown phenomena are observed, scientists find it natural to look for their cause and to devise models which will help in that effort. The process of organizing observations implies some model which provides a structure for the resulting list. This model may only be a vague concept of the relationship between the observed endpoint and changes in the system, or its environment which may effect that endpoint. It may be no more than an effort to fit mathematical relationship to the effect and one or more variables that are thought to be related to it, but even the act of selecting the related variables necessitates some thought about the underlying mechanisms.

The effect of ionizing radiation on biological systems has been found to be the result of an extremely complicated combination of physical, chemical and biological processes. Thus the primary function of many models of the effects of ionizing radiation on cells has been to aid in the organization of the data so that some pattern can be recognized in the complex collection of causes and effects. As the data is organized, trends become visible, and the model can be used to predict effects under conditions which have not

previously been tested experimentally. These predictions, which may be derived from purely conceptual models but which are more likely to be quantitative if the model is given a mathematical formulation, can be used to help in the design of experiments to test the validity of the assumptions of the model. Thus model building and testing becomes an iterative process. Refinements in the model provide new predictions, which can be used to devise new experiments, which produce new data, which provides the information needed to refine the model.

The use of models to predict effects under conditions which haven't been explored experimentally has an important practical application as well. Radiation exposure limits for the protection of radiation workers and the general public are based, as much as possible, on real human health effects data. However, those data are quite limited with respect to the range of doses, the variety of effects observed, and the statistical reliability. Thus it is necessary to utilize whatever models are available to guide the extrapolation to the effects of low doses and to predict possible effects under different conditions of exposure. To some extent data obtained with experimental animals can be used to estimate the effects in humans, but even animal experiments are limited to relatively high doses and dose rates by the cost and experimental difficulty involved in trying to obtain statistically reliable results at doses which are relevant to the majority of actual human exposures. Thus it is frequently necessary to devise models of the effects on experimental animals and then to devise an additional model that relates the animal response to health effects in humans.

The most important use of models may be their inherent relationship to the mechanisms which are responsible for the effects being modeled. The health effects of irradiation involve not only the production of biochemical damage by different radiations under different physical and chemical environments, but also the modification of that damage by a variety of biochemical systems which may be involved in modifying damage produced by other environmental insults and the normal metabolic activity of the cell. Furthermore, both the production of specific biochemical changes and the response of cells to those changes may depend on the current and past exposure of that cell to environmental factors which alter the levels of protective compounds and the activity of the repair systems. In order to interpret or predict interactions between radiation exposure and other health risk factors such as smoking and industrial chemical exposure, we must know considerably more about the mechanisms governing these effects.

BACKGROUND

Limitations on Models

All efforts to model natural systems face a dilemma. In order to describe the complex processes which occur in nature, the model must include many variables. However, to be testable and useful in any predictive way the model must not have more variables than the experiments which are used to test it. For example, a very simple target model is sufficient to describe the inactivation of phage by low LET radiations, but this is not very helpful when considering the risk to mammalian cells with their complex capacity to repair DNA strand breaks and to attenuate the killing effect during a post

irradiation holding period. On the other hand, a detailed model of mammalian cell survival involving coefficients for the production of half a dozen different forms of DNA damage and the repair kinetics for all of the known DNA repair systems would probably not be useful because there are no experiments which can be used to test so many variables simultaneously.

Much effort goes into devising models which can be evaluated using currently available experimental techniques. Thus as new experimental Southern blot analysis become available, new biological effects such as DNA fragment deletion have been modeled. These new phenomena, which can be included in the model, make it possible to test models which incorporate a wider range of features. A detailed model of mechanisms may require more parameters than can be tested, but by carefully choosing potential experiments it is possible to devise definitive comparisons between alternative models. Models with only two parameters can easily describe the dose effect relationship for acute exposures to low LET radiations, but the effects of dose-rate, split-dose, and delayed-plating schedules can be related to one or more repair processes to provide an opportunity to evaluate additional parameters. By considering the effects of oxygen and differences in LET on the probability of producing damage may make it possible to evaluate even more.

It is seldom, if ever, possible to design an experiment, or even a set of experiments, which proves a specific model. The most fruitful approach is generally to contrast two or more alternative models which predict a different and mutually exclusive effect under specific conditions and determine which of

the models are incorrect (Platt 1964 (1)). As the variety of models which have been shown to be incorrect increases, confidence in the alternative improves. Similarly it is more advantageous to test a variety of different models using one or more carefully studied biological systems, than to determine the number of different systems which behave in a way consistent with a specific model. In addition, testing large numbers of different systems provides information on which characteristics affect the parameters used in the model. This also may provide information on the sources of biological variability, and on how to control it. For example, it has long been known that the position of the cell in the cell cycle has a dramatic effect on the radiation sensitivity, so by limiting the experimental population to a specific age, i.e., specific point in the cell cycle, this source of biological variability is minimized and the effects of the parameter being tested can be seen more easily.

Attempts to limit biological variability and to obtain more precise measurements have often lead to the use of experimental conditions and, radiation doses which are far removed from the conditions which prevail in normal tissues at reasonable doses. The results obtained with such experiments are valuable in determining the nature and effectiveness of specific mechanisms, but may not be appropriate for the determination of the significance of those mechanisms at realistic doses. Evaluation of the significance of specific mechanisms is one of the primary challenges in modeling. Unusual biological systems or extreme doses may also provide the most powerful test of specific aspects of models, but such conditions may introduce competing processes which are not significant at the realistic

doses. In order to avoid testing models which are only relevant to the extreme situations, it is important that the model can also be confirmed at a dose where multiple energy deposition events are rare.

Phenomenological versus Mechanistic Models

It is always difficult to establish a boundary between phenomenological and mechanistic models; almost all involve some elements of both. The extremes are easy to identify. A few models are almost completely phenomenological having a minimum of underlying assumptions about mechanisms, while some are nearly entirely mechanistic with only a few parameters which must be determined by fitting to experimental data. However, the majority of models lie between these two extremes, with a significant basis in mechanistic assumptions and a phenomenological approach to fitting those mechanisms together into a complete picture. In preparing this and the accompanying section we have attempted to divide the known models in a logical fashion and minimize overlap. However, the division is often arbitrary and we do not claim that we can properly determine whether a specific model is mechanistic or phenomenological. In fact some features of specific models are mechanistic while other features are phenomenological. As a result, different aspects of a single model may be presented in both sections.

Biological endpoints

Reproductive death of individual cells, roughly following the procedures of Puck and Marcus (1956 (2)), is the most common endpoint described by

phenomenological models. By this endpoint we measure the fraction of cells which have not lost their potential for unlimited proliferation, i.e., cells which are capable of passing on genetic heritage through their progeny. This endpoint has been studied for many years, and is accessible for most types of cultured cells so that a great deal of experimental data is available. It is readily applicable to new types of experiments since the fraction killed can be measured over a wide range with relatively good statistical precision. Furthermore, when finally extrapolating to human health effects, it is important to understand the effects on cell growth potential so that other effects can be appropriately evaluated in the presence of the loss of cells from the population.

However, it is unlikely that cell killing is primarily responsible for the health effects of concern in radiation protection situations. Although cell killing is believed to be correlated with the deterministic effects of irradiation (protection levels are set so that those effects do not occur), the stochastic effects of radiation are believed to result from nonlethal modification of single cells within the animal. Thus the more relevant endpoints are malignant transformation, specific locus mutation, chromosome aberration, and other heritable genetic alterations. However, reproductive death, interphase cell death, and mutation marker salvage interfere with the measurement of these more relevant endpoints. It may be possible to argue that malignant transformation is the most important endpoint for the study of health effects, but malignant transformation is probably the endpoint of a long sequence of unidentified specific changes within the cell. Since many if not all of these changes are probably DNA deletions, rearrangements, or point

mutations, and since it is similar DNA changes which are usually responsible for cell death it can also be argued that for many tests of models, the endpoint used can be selected for experimental efficiency.

The Phenomena to be Modeled

A remarkable variety of factors effect the response of cells and more complex biological systems to ionizing radiation. Often the effect of these factors can provide additional information for the testing of the models, while in other situations these factors are of interest in their own right. The phenomena which have most often been included in models are the response to acute doses of low LET radiation, the relative biological effectiveness as a function of radiation quality (often expresses in terms of LET), the change in effect with change in dose rate, interval between two doses when the dose is split or the time between irradiation and the initiation and progression through the cell cycle by changing the culture conditions. The difference in the response of cells in different parts of the cell cycle at the time of irradiation, the effects of changes in the chemical environment such as the oxygen concentration or radical scavenger, and the effect on cell survival as a function of the time between irradiation and assay for cell survival have also been discussed. Although most models do not treat all of these phenomena explicitly, they normally cover a portion of them. The more phenomena that are described, the more precisely a model can be related to underlying mechanisms.

Every biological system has a unique response to radiation, but the differences are frequently only a matter of degree, with most systems having qualitatively similar response. Those systems with unique characteristics deserve special attention. Although the exception may be the result of an unusual adaptation to a specific environment or an unusual mutation during the adaptation of a cell line to continuous culture, it is often the exceptions to the rule which provide the critical information. However, we will limit this discussion to the typical response leaving the investigation of exceptions to others. Figure 1 illustrates the typical relationship between survival, dose, and dose rate for eukaryotic cells exposed to low LET radiations. The reduction in slope with decreasing dose rate is assumed to be the result from repair during irradiation. Here repair refers specifically to observed changes in survival (or other endpoint); any association between this and specific changes at the molecular level, e.g., double strand break rejoining, is still only an assumption. Repair is observed in other types of experiments as well. In Fig 2, split-dose repair occurs during the interval between exposures in a fractionated irradiation, while the delayed-plating repair occurs after the irradiation is completed. This difference becomes significant when some damage can become more effective at higher concentration; opportunity for repair during irradiation would then be more effective than repair time which occurred after the exposure. The effect of oxygen, the most common radiosensitizer, is illustrated in Fig 3. Oxygen seems to react with initial damage in such a way as to prevent the damage from undergoing a spontaneous change to an innocuous condition.

The cell-cycle effect for reproductive survival following low LET radiation is illustrated in Fig 4. Although this type of response is typical, there are many variations with different cell types and depending on experimental conditions, but both the sensitivity of DNA to damage and the ability to repair damage varies throughout the cell cycle. It should be remembered that the assay used to determine reproductive death is somewhat arbitrary. Irradiated cells do not simply continue to grow undisturbed, or to die. There is always a delay in cell progression, slow growth mutation, sectorized colonies, and delayed lethal effects which result in different measures of lethality with different assay procedures.

One of the most extensively studied phenomena in radiation biology is the effect of different radiation qualities. The typical response for cell survival is shown in Fig 5. However, radiation quality also alters the effects of repair, oxygen, radical scavengers, and cell cycle age. Due to the differences in the spacing of ionizations along the tracks of the charged particles involved, different radiations can produce effects which differ qualitatively as well as quantitatively.

MODELS FOR REPRODUCTIVE SURVIVAL

Radiation Quality models

The effects of changes in radiation quality and the methods for characterizing that elusive property have dominated much of radiation biophysics modeling. Not only is the quality factor a major concern in

radiation protection, but there is obviously something of fundamental importance involved when the response of cells to the same amount of energy can vary by a factor of 20 or more depending on the velocity of the particles that deposit the energy. One can approach the effects of changes in the radiation alone by using experimental data for a limited set of radiations to establish the response of cells as a function of selected characteristics of those radiations, and then use measured or calculated values of those characteristics for additional radiations to predict the response.

One highly successful model of this type was proposed by Katz and coworkers (1967 (13)) and has been extended to cover a wide range of radiations (Katz 1971 (4), Katz 1985 (5)). In this model it is assumed that the response of any small radiation detector, including a DNA molecule or a cell, to high energy ion irradiation can be divided into a response to the interaction of the ion with the target (known as ion kill) and the interaction of the delta rays with the target (known as gamma kill). Four parameters, σ_0 , κ , m , and D_0 , are used to describe the cell. D_0 comes directly from the final slope of the survival of cells exposed to gamma rays and the other parameters are determined by fitting the survival expression

$$S = e^{-\sigma D L} \{ 1 - [1 - e^{-(1-P)(D/D_0)}]^m \} \quad (1)$$

where $\sigma = \sigma_0 (1 - e^{L+2/\kappa\beta})^m$

$P = \sigma/\sigma_0$

$D =$ absorbed dose

$L =$ stopping power

Z^* = effective charge on the charged particle

β = the velocity of the particle relative to that of light

to the experimental data for a few radiation types. Clearly, the same irradiation conditions, specifically single exposure acute irradiation, must be used in all of the experiments to determine the parameters, and the model only applies to similar high dose rate exposures. Consequently, this model deals only with the effects of radiation quality and makes no attempt to deal with other phenomena such as dose rate effect or repair after irradiation. Using parameters which were determined soon after the model was originally formulated it has been quite successful in predicting the response of cells to new radiations as experiments with new accelerators have been performed. Figure 6 shows the fit obtained for mammalian cells exposed to a variety of ions. The relationship of this model to some others has been explored by Curtis (1989 (6)) who showed that it becomes linear-quadratic in form if $m=2$.

Another approach to determining the effects of radiations of different qualities is based on the assumption that the response of a cell to irradiation depends on the energy deposited in a small volume such as the cell nucleus. Although the actual volume which is important in determining the response is not known, it is assumed that it can be simulated by a sphere a few micrometers in diameter. The energy deposited, which can be expressed as the microdosimetric quantity, y (the energy deposited divided by the mean chord length of the sample volume), is then a reasonable indicator of radiation quality. Several different approaches have been taken to evaluate the relationship between lineal energy and probability of effect. Initially,

Varma and Bond (7,8) limited the analysis to low-dose or low dose-rate experimental data where the observed biological effect was the product of the interaction of a single charged particle track, and could be related to the single event distribution, y . They assumed a relatively simple relationship between y and effectiveness, and were able to evaluate the coefficients of the relationship for *tradescantia* mutation using data obtained for radiations with a wide range of LET spectra. Later Varma and coworkers (1985 (9)) showed that the hit size effectiveness function determined in this way could be used to predict the effects of high doses of low LET radiation delivered at high dose rate so that biological repair need not be considered. Morstein and coworkers (1989 (10)) and Zaider and Brenner (1985 (11)) took more flexible approaches to determining the effective relationship, avoiding the need to assume general form of the relationship. The resulting effectiveness functions for a wide range of cell types and endpoints generally resemble Fig 7 (derived from Morstin).

The basis of this approach is the assumption that the spatial distribution of energy deposition within the cell nucleus is not critical in determining the effect, and the elimination of the effects of repair by limiting the application to single event effects or high dose-rate effects where repair does not occur. Within these limitations, this approach has been applied to a variety of cell types and endpoints such as chromosome aberration as well as cell survival. Its applicability to low dose effects makes it particularly relevant to the needs of radiation protection. The results obtained for different cell types and by different mathematical approaches are remarkably consistent. The differences between cell lines suggest that this

approach may help in identifying those characteristics of cells which are related to their response to radiation.

Cell Response Models

The second major grouping of models involves those which attempt to describe the response of cells in terms of the probability of energy being deposited, the organization of the sensitive material in the cell, and the response of the cell to specific concentrations of damage. The initial goal of these models is to describe the shape of the acute-exposure dose-response curve, with refinements to add dose-rate and fractionation effects, and RBE. There are many different models in this group, ranging from quite simple, to those with a substantial involvement of biological processes, but they are all based on the same basic assumptions and are all constrained by the consequences of those assumptions. Although most of the models have been formulated in terms of reproductive survival of cells, the underlying assumptions deal with effects in general. The models may be applicable to other endpoints as well. However, the specific mechanisms involved may be different for each endpoint, and the coefficients derived from a model for cell survival may not be applicable to mutation or a specific step in malignant transformation. Models of the mechanisms leading to these specific endpoints may eventually indicate the relationships between endpoints, making it possible to predict mutation and transformation rates from survival data, or it may be necessary to fit a model directly to data for each endpoint.

A reasonable starting point for a model of radiation effects on single cells is to assume that if a charged particle passes through some region, usually referred to as the target, there is a finite probability of doing critical damage. If this probability is assumed to be the same for all charged particle events, either because the radiation is controlled so that all events are identical or because the cell is not sensitive to differences in the energy deposition, and all events act independently in the cell, then from Poisson statistics the probability that there will be one or more critical events in the cell is

$$f=1-e^{-\lambda D} \quad (2)$$

where λ is the mean number of critical hits per cell per unit dose, D . Since the probability of a cell surviving irradiation is equal to the probability of it not having lethal damage,

$$S=1-f=e^{-\lambda D} \quad (3)$$

There are some system, such as enzymes irradiated in the dry state and some simple viruses, where the only significant type of damage is a single ionization at a critical location, and the conditions required for the validity of the above equations are met. However this is not the case for most biological systems. It seems unlikely that an isolated ionization in a large molecule would be as likely to do "critical" damage as would a cluster of closely spaced ionizations which would disrupt more bonds and possibly produce larger distortions in the higher level structure of the molecule. The

increase in relative biological effectiveness with increasing LET of the radiation is consistent with this assumption. Furthermore, it seems unlikely that the products of separate charged particle events within a single target would act entirely independently. Cells are known to repair most radiation induced damage, but the capacity to repair is probably not unlimited. It is also likely that the process of replication prevents further repair so there will be competition between repair and replication of damaged sites. The process of repair requires information on the "correct" structure but as the concentration of damage increases, the chance that this information will be lost increases. Damaged DNA molecules may undergo interactions, such as formation of exchanges or deletions, which result in damage which is less easily repaired. These, and probably many other types of interaction, cause an increase in effect, but such interaction may also decrease the effectiveness of damage. For example, some cell types are known to activate additional repair systems in response to a damage. The effects of multiple track processes can be included by a simple polynomial expansion of equation 2

$$S = \exp-(a_1D + a_2D^2 + a_3D^3 \dots) \quad (4)$$

Most of the models which have been developed in recent years incorporate some combination of features such as repair of sublesions and interaction which blocks repair. The diversity of these combinations makes a comparison of models or a progression from basic models to more advanced versions quite complex. However, many models share specific features and these models can be grouped according to shared features, even though each model may also possess other differences which give it quite distinct characteristics.

One of the major differences between phenomenological models in this group has been the assumption made about the nature of the interaction process which is assumed to be responsible for the curvature of the dose response relationship. It has generally been assumed, based primarily on Elkind's work on the split-dose recovery effect (Elkind and W 1967 (12)), that the shoulder of the survival curve, the dose-rate effect, and repair of damage are interconnected. However, there has been considerable controversy about the nature of the connection. The difference in the alternative assumptions about interaction has been expressed in terms of the effectiveness of unrepaired damage produced by a single charged particle track. Some models assume that the damage produced by a single (low LET) charged particle track is insufficient to produce an effect and that the damage produced by two or more tracks must interact in order to produce the effect. This sublethal damage is generally assumed to be repaired, but if the damage does not interact it makes no difference whether it is repaired or not, it will not be detectable in terms of an effect on the cell. The alternative approach assumes that damage produced by a single low LET track is lethal to the cell unless it is repaired, and that the interaction is some type of process which limits the amount of repair or its accuracy.

If some damage is assumed to be sublethal in nature, then there are at least two different ways in which the interaction might come about. Several early models (Swann and del Rosario 1934 (13), Kellner and Hug 1963 (14), Dienes 1966 (15), Rajewsky and Danzer 1934 (16), Lea 1938 (17)) assume what amounts to a target with two damaged states, sublethally damaged and lethally damaged. Sublethally damaged cells must receive additional damage to be

converted to lethally damaged, but they are also subject to repair which converts them back to undamaged cells. For example the expression for cell survival for the Swann-del Rosario model is

$$S=[R_1 \exp(-R_2 D) - R_2 \exp(-R_1 D)]/(R_1 - R_2) \quad (6)$$

with

$$R_1, R_2 = (1/2)[K_1 + K_2 + ((K_1 + K_2 + K)^2 - 4K_1 K_2)^{.5}] \quad (5)$$

where K_1 is the rate of cell damage, K_2 is the rate of damaged cell killing T is the mean recovery time for damaged cells, and K is $(D'T)^{-1}$ where D' is the dose rate.

The alternative assumption is that is that bits of damage, sometimes referred to as sublesions, are produced by radiation interactions and are repaired by a process which has a fixed rate for each bit of damage. The damage accumulates in the cell with increasing dose until an equilibrium which depends on the dose rate is reached. New damage has a probability of interacting with existing damage to produce a lethal effect which is proportional to the concentration of damage. Models of this general type were developed by Roesch (1967 (18)), Kelllerer and Rossi (1972 (19)), and Payne and Garrett (1975 (20)) and lead to the expression for survival

$$S = \exp[-CD - A(D'\tau)^2(\exp[-D/(D'\tau)] - 1 + D/(D'\tau))] \quad (7)$$

where C and A are coefficients for single event and two event damage respectively. The characteristic time, τ , the mean time required to repair a lesion.

Both of these versions of the sublethal damage repair model can be made to fit typical survival curves to within the precision of the experimental measurements yet they differ in a fundamental way. Equation 7 suggests plotting dose rate data as $-\ln S/D_2 - C/D$ versus the duration of the irradiation, D/D' (Braby and Roesch 1978 (21)). When this is done the accumulation models result in a single curve independent of the dose rate, while the promotion models result in families of curves. Experimental evidence for a variety of mammalian cell lines (Metting, et al., 1985 (22)) clearly shows the single curve rather than a family, Figure 8, when stationary phase cells are exposed to a wide variety of dose rates.

An important parameter in models which describe dose rate and fractionation experiments is the mean time required to repair a lesion. This time can be obtained from the $-\ln S/D^2$ curve, but it requires a lot of low dose rate irradiations to obtain τ with reasonable precision. It is much easier to obtain data for a wide range of repair times by using split-dose methods. The accumulation model can be solved for an irradiation which consists of two fractions given at high dose rate and separated by a variable interval t_r (Braby et al (23)), Nelson et al 1980 (24)). This analysis shows that the repair time can be determined from the slope of a plot of $-\ln S_m/S$ versus t_r , where S_m is the maximum survival. The model assumes that any repair which might occur after the second irradiation is constant. In an attempt to meet

this requirement, cells are plated immediately after the second dose. Experimental results for Chlamydomonas reinhardtii, a eukaryotic algae which is amenable to determination of τ by the analysis of dose-rate data, showed that split-dose and dose-rate experiments resulted in the same repair rate. When the repair rate of plateau-phase CHO cells was determined (Nelson 1990 (25)) the results, Fig 9, indicate that a model with a single repair process is too simple to describe the actual biological situation. Plateau-phase cells which could be held for three days with less than 0.1% contamination of cycling cells were used in order to obtain a good value for S_m . Dose rates as high as 10 Gy/sec and intervals ranging from 10 sec to 24 hours were used in order to detect the widest possible range of repair rates. The majority of the damage was found to be repaired with a mean time of about 68 minutes, with an additional component being repaired with a characteristic time of about 18 hours. No repair with a characteristic time between 10 sec and 50 minutes could be detected.

The split-dose results suggest that at least two distinct types of damage are being repaired, and that both of them involve some form of interaction so that expression as reproductive death becomes more likely with increasing dose. However, many different types of interaction might be responsible for the observations, and the models discussed so far have only dealt with one, the interaction of sublethal damage to produce lethal or potentially lethal damage. The main alternative to this type of model assumes that all relevant damage is lethal unless repaired. This approach follows naturally from the observation of repair in delayed plating experiments (Phillips & Tolmach 1966 (26)), a phenomena which is not predicted by the sublethal damage models.

However, if there is a fixed probability for repair, this does not change the shape of the survival curve. Several different approaches can be taken to include a decrease in efficiency of repair with increasing dose. One approach, used by a number of authors (Haynes 1966 (27) and 1975 (28), Pohlit 1975 (29), Kappos and Pohlit 1972 (30), Garrett and Payne 1978 (31)), essentially assumes that radiation can inactivate repair enzymes as well as damage the structures that they repair. Thus, as the dose increases, repair becomes less efficient due to a reduction in the repair system. A closely related approach is to assume that irradiation reduces the supply of energy (ATP) within the cell, thus reducing the effectiveness of repair (Haynes 1966, (27) Pohlit 1981 (32)). Although the depletion of ATP has not been found to be sufficient to account for the apparent reduction in repair in any system, the depletion of repair enzymes is consistent with the data for simple systems with relatively small target volumes and small amounts of repair enzyme. For more complex mammalian cells, the effect of interaction is evident at a few Gray which would require that the repair enzymes themselves must be very large. A more realistic alternative is the assumption that the supply of repair enzyme is quite limited in ordinary cells, and that at high doses the repair system is saturated. This approach was developed by Goodhead (1980 (33), 1985 (34)) with the further assumption that repair molecules are not recycled, and leads to the expression

$$S = \exp -p(aD - c_0) / \{1 - (c_0/aD) \exp[kt(c_0 - aD)]\} \quad (8)$$

where a gives the number of potentially lethal lesions per unit dose, c_0 is the initial number of repair molecules, p is the fraction of the damage which

is capable of producing the specific biological effect, and k is the rate constant for repair. This model provides good fits to acute survival data, Fig 10 , and delayed plating experiments but requires an additional component, the rate of replacement of repair molecules, in order to apply to dose-rate or split-dose experiments.

One consequence of the type of interaction which is characterized by repair saturation models is that the total amount of repair which occurs depends on the amount of damage, the rate of replenishment of the repair material, and the total time for repair. It is not a function of the time dependent concentration of the damage. This is significantly different from the accumulation models and some mechanistic models (Curtis 1986 (35)) where the total amount of repair which can occur is reduced by interactions which depend on the square of the concentration of damage. This difference can be tested by comparing the survival of cells receiving the same total dose in a split- dose experiment or in a delayed-plating experiment. Results for this type of comparison for plateau-phase CHO cells is shown in Fig 11. The split-dose exposure results in higher final survival, even though cells were plated immediately after the second dose. If the cells exposed to the split dose are held for a few hours after the second dose their survival is still higher. Thus this experiment indicates that the process which is responsible for the shoulder of the survival curve is not simply the saturation of a repair process; it may be any of the interaction processes which becomes more probable with increasing concentration of the damage.

Those models which are consistent with the split-dose versus dose-rate data for CHO cells include the accumulation models and some potentially lethal damage models. These two types of models differ in a fundamental way which will be important in the future development of mechanistic models as well as in the prediction of effects at low doses and low dose rates. The essence of the difference is whether the interaction process converts relatively innocuous damage to potentially lethal damage or whether it alters the probability that potentially lethal damage will be repaired. If the damage is sublethal, dose fractionation will become less effective with decreasing dose, i.e., decreasing concentration of damage. This is because unrepaired sublethal damage can not be detected. However, if the damage is potentially lethal, delayed plating will become more effective with decreasing dose because there will be less of the interaction that can block repair. In order to distinguish between these possibilities the total change in survival due to repair as a function of dose is needed. However, cells are known (Curtis 86 (35)) to repair damage for about three hours after replating in growth medium, so delayed plating experiments do not detect all of the damage being repaired. Drugs such as β -ara-A are known to block repair, but may also alter the configuration of the sensitive molecules and thus alter the production of damage if they are present during irradiation. Recent experiments in which the repair inhibitor was added after the irradiation, Fig 12, show an exponential survival curve for repair inhibited cells, and a corresponding increase in the effectiveness of repair with decreasing dose. If this result is representative of the situation for other cell types and for cells in other culture conditions, it suggests that biochemical mechanisms which can be characterized as resulting from the interaction of sublethal damage to produce

lethal damage play a very small part in the effect of radiation at low doses. However, mechanisms which can be characterized as altering the repairability of potentially lethal damage or the effectiveness of repair, may play a major role in the response at low doses.

MODELS FOR OTHER ENDPOINTS

Chromosome Aberrations

The development of models for the production of chromosome aberrations as a function of radiation quality has roughly paralleled the development of models for cell survival. A model based on the interaction of sublesions was developed by Neary (1965 (36)). In this model the sublesions are assumed to be produced by a single ionization and interaction is assumed to occur within a relatively small site within the nucleus. Many, possibly overlapping, sites exist in each nucleus. The probability of an aberration, Y , is

$$Y = NE\{1 - \exp(-mgk^2)[1 - \{1 - \exp(-mk + mgk^2)\}^2]\} \quad (8)$$

where N is the number of sites in the nucleus, E is the probability of sublesions within the site interacting, m is the mean number of tracks through the projected area, A , of the chromosome segment within a site, g is the probability that a track which has traversed one target chromosome of a site will also traverse the other, and k is the probability that one track through a target produces a primary lesion, a function of p , the probability that a single energy loss event in a target chromosome produces a sublesion and of

the LET. This model can be used to predict the general shape of the curve representing the probability of producing an interchange by a single track interaction or through interaction of two tracks as a function of LET Fig 13. However, due to the large number of parameters in the model it is necessary to assign mechanistic significance to them and to try to evaluate them independently in order to test the validity of this approach.

More recently, the success of repair depletion and related models for cell survival has suggested that the interaction of sublesions may not be required for chromosome aberration (Cornforth 1990 (37)). However, the experimental evidence does not seem to be consistent with a single hit mechanism at low LET.

Oxygen Effect

The dependence of the oxygen enhancement ratio, shown in Fig 3, on oxygen concentration has been studied extensively. Alper and Howard-Flanders (38) proposed the relationship

$$\text{OER} = (m[\text{O}_2] + k)/([\text{O}_2] + k) \quad (9)$$

where m and k are constants and $[\text{O}_2]$ is the oxygen concentration. As an understanding of radiation chemistry of cells developed (Quintiliani 1986, (39)) Chapman and Koch 1986 (40)) it became generally accepted that oxygen reacts with radiation-induced DNA radicals to produce products which are more damaging than those which would otherwise result. Kiefer (1975 (41)) showed

that if a single type of initial damage is converted into either type x if it reacts with oxygen, or type y if it reacts with a hydrogen donor such as glutathione, with rates k_x and k_y respectively and these two types of damage are repaired by processes with rates k_{xo} and k_{yo} or converted to lethal damage with rates k_{xx} and k_{yy} then the constants in equation 9 become

$$k = k_y/k_x \quad (10)$$

and

$$m = (1 + k_{yo}/k_{yy})/(1 + k_{xo}/k_{xx}) \quad (11)$$

This provides a starting point for an understanding of the mechanisms involved in the repair of damage formed in the presence and absence of oxygen, but as Chapman and Koch pointed out (1986 (40)), there are likely to be many different DNA radicals formed, and there may be different repair processes involved as a result. Alper (1984 (42)) has shown that if more than one type of oxygen dependent damage does exist it will be very difficult to demonstrate it on the basis of OER measurements.

FUTURE DIRECTIONS

The comparison of model predictions with experimental results has lead to a significant evolution in our thinking about the nature of the response of biological systems to ionizing radiation. Attempts to model the relative biological effectiveness of different radiations have dramatized the importance of the stochastics of energy deposition at the cellular and subcellular level. Tests of dose response relationships under different

irradiation conditions have shown that the modification of the effectiveness of damage by cellular processes is a major factor in determining the final effect. Modeling the oxygen effect has shown how a single type of initial damage can result in different DNA alterations, and how differences in the rates of repair of these alterations can change cell survival. It is probably not possible to change any physical property of the irradiation without some corresponding change in the biological modification of the resulting damage.

Models of the survival of cells are only one step in understanding the health effects of ionizing radiation, but an important step since reproductive death is probably the result of some of the same processes which lead to malignant transformation and other undesirable changes in growing cells. Refinements are still needed in models of the reproductive survival in order to determine the role of multiple repair processes and to understand the long term effects of repaired cells in the population. Models now include the assumption that "repaired" DNA is the same as undamaged DNA, but there is some evidence (Seymour and Mothersill 1989 (43)) that additional changes occur many cell generations later, or that repair only converts damage to a form that cannot be detected for many generations. However enough is now understood about cell survival that we should be able to apply similar modeling concepts to the individual steps in the complex process of transformation. Due to the limited number of variables which can be tested in realistic biological experiments, it is likely that different cell types and transformation assays will be needed to investigate each step in a multistep process. This will be difficult, because there is conflicting evidence about the number of steps involved in carcinogenesis, and it may be that different combinations of

changes are required to produce transformation in cells of different tissues. However, it is reasonable to speculate that DNA deletions, rearrangements, and point mutations may all play roles in different steps of the process. It may also be possible for radiation to effect DNA indirectly through damage to molecules involved in critical feedback mechanisms or through damage to structural components of the cell which are involved in regulation of growth or expression of specific genes.

Both the degree of understanding of the phenomenology of radiation effects and the availability of biochemical techniques for detecting the rare changes produced in cells by ionizing radiation make it practical to attempt to identify the specific mechanisms involved in radiation damage and repair. Several chemical repair processes, for example double strand break rejoining, have been studied extensively, but it is difficult to establish quantitative relationships between them and endpoints such as mutation. It is likely that there are several steps between damage to DNA and the expression of that damage as a deletion or rearrangement. The fact that some phenomenological models can be rejected reduces the range of mechanisms which have to be considered for these steps. The results discussed above suggest that we should be looking for mechanisms which alter the effectiveness of repair, rather than ones that might convert innocuous damage into a more serious form. However, they still indicate that this alteration of repair effectiveness must occur over distances comparable to the diameter of the nucleus of the cell. This probably eliminates mechanisms such as interaction of damage at different points on a single DNA molecule, but leaves open many other possibilities for

mechanisms involving the information encoded in the DNA and in its structural relationship with the rest of the cell.

ACKNOWLEDGEMENTS

Work supported by the Office of Health and Environmental Research (OHER),
U. S. Department of Energy under Contract DE-AC06-76RLO 1830.

References

1. J. R. Platt, Strong Inference, Science, 146: 347-353 (1964)
2. T. T. Puck and P.I. Marcus, Action of X-rays on Mammalian Cells, J. Experimental Med., 103:653-666 (1956)
3. J. J. Butts and R. Katz, Theory of RBE for Heavy Ion Bombardment of Dry Enzymes and Viruses, Radiat. Res., 30:855-871 (1967)
4. R. Katz, B. Ackerson, M. Homayoonfar, and S. C. Sharma, Inactivation of cells by Heavy Ion Bombardment, Rad. Res., 47: 402-425 (1971)
5. R. Katz, D. E. Dunn and G. L. Sinclair, Thindown in Radiobiology, Rad. Protect. Dosimetry, 13: 281-284 (1985)
6. S. B. Curtis, The Katz Cell-Survival Model and Beams of Heavy Charged Particles, Nucl. Tracks Radiat. Meas., 16:97-103 (1989)
7. V. P. Bond and M. N. Varma, Low Level Radiation Response Explained in Terms of Fluence and Cell Critical Volume Dose, in Proceedings of the Eighth Symposium on Microdosimetry, Julich, West Germany, Harwood Academic, London: 423-437 (1982)
8. M. N. Varma and V. P. Bond, Empirical Evaluation of a Cell Critical Volume Dose vs. Cell Response Function for Pink Mutations in Tridescantia, in

Proceedings of the Eighth Symposium on Microdosimetry, Julich, West Germany, Harwood Academic, London: 439-450 (1982)

9. M. N. Varma, V. P. Bond and G. Matthews, Hit-Size Effectiveness Theory Applied to High Doses of Low Let Radiation for Pink Mutations in *Tridescantia*, Rad. Protection Dos., 13: 307-309 (1985)

10. K. Morstin, V. P. Bond, and J. W. Baum, Probabilistic Approach to Obtain Hit-Size Effectiveness Functions which Relate Microdosimetry and Radiobiology, Rad. Res., 120: 383-402 (1989)

11. M. Zaider and D. J. Brenner, On the Microdosimetric Definition of Quality Factors, Rad. Res., 103: 302-316 (1985)

12. M. M. Elkind and G. F. Whitmore, The Radiobiology of Cultured Mammalian Cells, Gordon and Breach New York (1967)

13. W. F. G. Swann and C. del Rosario, The Effect of Radioactive Emanations Upon *Euglena*, J. Franklin Inst., 211: 303-317 (1934)

14. A. Kellerer and O. Hug, Zu. Kinetik der Strahlenwirkung. Biophysik, 1: 33-50 (1963)

15. G. J. Dienes, A Kinetic Model of Biological Radiation Response, Rad. Res., 28: 183-202 (1966)

16. B. Rajewsky and H. Danzer, Über einige Wirkungen von Strahlen. VI. Eine Erweiterung der Statistischen Theorie der Biologischen Strahlenwirkung, Z. Physik., 89: 412-420 (1934)
17. D. E. Lea, A Theory of the Action of Radiations on Biological Materials Capable of Recovery. I. The Time-Intensity Factor, Br. J. Radiol., 11:489-497 (1938)
18. W. C. Roesch, A Model for the Action of Radiation on Simple Biological Systems, in Proceedings of the First International Symposium on the Biological Interpretation of Dose from Accelerator-Produced Radiation, CONF-670305: 297-305 (1967)
19. A. M. Kellerer and H. H. Rossi, The Theory of Dual Radiation Action, Curr. Top. Radiat. Res. Q., 8: 85-158 (1972)
20. M.C. Payne and W.R. Garrett, Some Relations Between Cell Survival Models Having Different Inactivation Mechanisms, Rad. Res., 62: 388-394 (1975)
21. L. A. Braby and W. C. Roesch, Testing of Dose-Rate Models with Chlamydomonas reinhardtii, Rad. Res. 76: 259-270 (1978)
22. N. F. Metting, L. A. Braby, W. C. Roesch and J. M. Nelson, Dose-rate Evidence for two Kinds of Radiation Damage in Stationary Phase Mammalian Cells. Rad. Res., 103: 204-218 (1985)

23. L. A. Braby, J. M. Nelson, and W. C. Roesch, Comparison of Repair Rates Determined by Split-Dose and Dose-Rate Methods, *Rad. Res.*, 82: 211-214 (1980)
24. J. M. Nelson, L. A. Braby and W.C. Roesch, Rapid Repair of Ionizing Radiation Injury in *Chlamydomonas reinhardtii*, *Rad. Res.*, 83: 279-289 (1980)
25. J. M. Nelson, L. A. Braby, N. F. Metting, and W.C. Roesch, Multiple Components of Split-Dose Repair in Plateau-Phase Mammalian Cells: A New Challenge for Phenomenological Modelers, *Rad. Res.*, 121: 154-160 (1990)
26. R. A. Phillips and L. J. Tolmach, Repair of Potentially Lethal Damage in X-Irradiated HeLa Cells, *Rad. Res.*, 29: 413-432 (1966)
27. R. H. Haynes, The Interpretation of Microbial Inactivation and Recovery Phenomena, *Rad. Res.*, S-6: 1-29 (1966)
28. R. H. Haynes, The Influence of Repair Processes on Radiobiological Survival Curves, In *Cell Survival after Low Doses of Radiation* ed by T. Alper, Institute of Physics/ Wiley, London: 197-208 (1975)
29. W. Pohlit, The Shape of Dose-Effect Curves for Diploid Yeast cells Irradiated With Ionizing Particles, In *Cell Survival After Low Doses of Radiation* ed. T. Alper, Institute of Physics/ Wiley, London: 190-196 (1975)

30. A. Kappos and W. Pohlit, A cybernetic Model for Radiation Reactions in Living Cells. I. Sparsely-Ionizing Radiations; Stationary Cells, Int. J. Radiat. Biol., 22: 51-65 (1972)
31. W. R. Garrett and M. G. Payne, Applications of Models for Cell Survival: The Fixation-Time Picture. Radiat Res., 73: 201-211 ((1978)
32. W. Pohlit and I. R. Heyder, The Shape of Dose-Survival Curves for Mammalian Cells and Repair of Potentially Lethal Damage Analyzed by Hypertonic Treatment, Rad. Res., 87: 613-634 (1981)
33. D. T. Goodhead, Models of Radiation Inactivation and Mutagenesis, In Radiation Biology in Cancer Research, ed. R. E. Meyn and H. R. Withers, Raven Press, New York: 231-247 (1980)
34. D. T. Goodhead, Saturable Repair Models of Radiation Action in Mammalian Cells, Rad. Res. 104: S-58 - S-67 (1985)
35. S. B. Curtis, Lethal and Potentially Lethal Lesions Induced by Radiation - A Unified Repair Model, Rad. Res., 106: 252-270 (1986)
36. G. J. Neary, Chromosome Aberrations and the Theory of RBE 1. General Considerations, Int. J. Rad. Biol., 9: 477-502 (1965)
37. M. N. Cornforth, Testing the Notion of the One-Hit Exchange, Rad. Res. 121: 21-27 (1990)

38. T. Alper and P. Howard-Flanders, Role of Oxygen in Modifying the Radiosensitivity of E. Coli, Nature, 178: 978-979 (1956)
39. M. Quintiliani, The Oxygen Effect in Radiation Inactivation of DNA and Enzymes, Int. J. Radiat. Biol., 50: 573-594 (1986)
40. J. D. Chapman and C. J. Koch, Comment on the paper by van der Schans et al., Int. J. Radiat. Biol., 50: 467-470 (1986)
41. J. Kiefer, Theoretical Aspects and Implications of the Oxygen Effect, Radiation Research ed by O. F. Nygaard, H. I. Adler and W. K. Sinclair Academic, New York: 1025-1037 (1975)
42. T. Alper, Adding Two Components of Radiosensitization by Oxygen, Int. J. Radiat. Biol., 46: 569-585 (1984)
43. C. B. Seymour and C. Mothersill, Lethal Mutations, the Survival Curve Shoulder and Split-Dose Recovery, Int. J. Radiat. Biol., 56: 999-1010 (1989)
x M. N. Varma and V. P. Bond, Hit-size effectiveness Approach in Biophysical Modeling, in Quantitative Mathematical Models in Radiation Biology. New York: Springer-Verlag: 119-124 (1988)

Figure Captions

Figure 1. An example of the typical dose and dose rate response of mammalian cells, in this case plateau phase CHO cells exposed to 250 KV x-rays.

Figure 2. Cell survival typically increases with time held in stationary phase after irradiation. The shoulder of the survival curve is recovered between doses in a split dose experiment.

Figure 3. Cells irradiated in air and in N_2 at low oxygen concentration seem to have the same response, with just a change in the effectiveness of the radiation.

Figure 4. Cell cycle position can cause large changes in the survival following a fixed radiation exposure (12).

Figure 5. Changes in charged particle velocity and charge have a dramatic effect on shape of the survival relationship as well as the sensitivity.

Figure 6. The Katz model provides fits to charged particle irradiation data based on the response of the cell to low LET irradiation and a fit to data for other high LET particles (4).

Figure 7. Hit size effectiveness functions are similar for endpoints such as HPRT mutations in human fibroblasts chromatid exchanges, and abnormal metaphase in CH2B2 hamster cells (10).

Figure 8. When plotted in a way which allows discrimination between models assuming cells are promoted from undamaged to damaged to lethally damaged states and models which assume that damage accumulates in cells, the data for plateau phase CHO cells are consistent with the accumulation assumption.

Figure 9. Split dose data for plateau phase CHO cells shows two repair rates, indicating two types of damage which differ in some way which alters their repair.

Figure 10. The repair saturation hypothesis is consistent with survival data for a widely different radiations (34).

Figure 11. Split dose and delayed plating data show higher final survival in the split dose case, where the damage concentration is relatively lower, the opposite of the prediction of repair saturation models.

Figure 12. Plateau phase CHO cells treated with ara-A for 4 hours starting immediately after irradiation show an exponential survival curve, compared to the shouldered curve resulting from 24 hour plating delay or from immediate plating in growth medium.

Figure 13. The LET dependence of the yield of chromosome aberrations produced by single tracks and by two track interactions can be predicted qualitatively (36).

SURVIVAL CURVES AT VARYING LET VALUES

END

DATE FILMED

01 / 02 / 91