Stochastic Models of Lesions Induction
and Repair in Yeast

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Abstract

In the past decade several stochastic models for the effects of radiation on cell survival have been proposed. We survey them briefly and consider their possible application to some experimental results of M. Frankenberg-Schwager and coauthors on irradiated yeast. One possible model is a slight modification of the model proposed by Yang and Swenberg [15]. It is shown that the modified model does not actually fit well and that the repair mechanism requires additional complications for adequate description.

1. Introduction

The present paper originated in an attempt to fit stochastic models of the effects of radiation on cells in culture to experimental data. There are several such stochastic models. They can be classified in three or four broad categories described below (Section 2). Most of the available experimental data has been in the form of dose response survival or transformation curves. A different kind of data was obtained by Dr. Frankenberg-Schwager and colleagues. They estimated the number of double-strand DNA breaks induced by the radiation and followed their repair under various circumstances. A graph published in [5], fig 7 and reproduced below attracted our attention as it seemed to be difficult to reconcile with the standard assumptions of the conventional stochastic models. It first appeared that the graph in question would be compatible with a modification of the model proposed by Yang and Swenberg [15]. Upon further examination it was shown that the proposed modification of the Yang-Swenberg model does not fit, or at least does not fit well. Some other explanations are needed. Some possibilities are described in Section 5.

2. Classification of the stochastic models

It is a recorded fact that if one plots the logarithm of surviving fraction of cells against radiation dose one obtains two different types of curves. One kind is linear in the dose. It occurs for high LET radiation or for circumstances in which the repair mechanism of the cell has been disabled [7] [11]. Another type, occuring for low LET radiation with repair, is a concave function of the radiation dose, exhibiting what is

called a "shoulder". (For terms used here without explanation, see the paper by Yang and Swenberg [15].)

Various explanations for the shoulder curves have been offered. One possibility is the "dual action theory" of Kellerer and Rossi [10].

It explains the shoulder through fluctuations in local energy deposition that create "sublesions", which, if close enough, can interact to lead to the actual biological lesions. According to such a theory, the interaction occurs essentially at the time of irradiation. A related explanation can be found in the book [2] by Chadwick and Leenhouts where they describe their so called (α, β) theory.

The same sort of explanation, albeit in a more subtle fashion, is described in the Yang-Swenberg paper [15].

A totally different explanation is given by Dudley T. Goodhead [9]. He attributes the shoulder to saturation of the repair mechanism. For the case of yeast, this is discussed, and rejected in the papers [5] and [7] by Frankenberg-Schwager and co-authors.

By contrast C. Tobias [14] and his colleagues assume that the average number of lesions is proportional to radiation dose and that the shoulder effect arises from interactions of lesions due to the repair mechanism. Tobias model was only partially stochastic. A fully stochastic version was given by Albright. A far reaching extension has now been given by R. Sachs [13].

There is considerable evidence that the "lesions" that prove lethal to cells are DNA double-strand breaks (DSB). In fact, one can argue that a single DSB left unrepaired or misrepaired will inactivate the cell [5]. Single strand breaks are considerably more numerous than DSB's, but they get repaired very rapidly. However thee have been claims that the actual lethal lesions are chromatin breaks in which the protein support of the DNA also gets broken. The Yang-Swenberg model can also be interpreted by saying that, as the radiation dose increases, the supporting structures are weakened and make DSB more probable. There is very little reason to believe that, generally, the interaction phenomena leading to shoulders are interactions of the track ends of the incident primary particles, even though such a proposal has been made and even though for the yeast experiments to be described shortly, one of the authors say that for irradiation by 30 Mev electrons and doses above 1000 Gy interaction is a possibility, (D. Frankenberg [3], page 323). See also the explanations of the differences between irradiation at low dose rate and at high dose rate in [6], page 715.

3. Counting DSB's in irradiated yeast

One of the difficulties with double-strand breaks (DSB) is that they cannot be readily seen or counted. In spite of this, experiments of M. Frankenberg-Schwager and co-authors [3], [5], [8], were designed to estimate the number of DSB's. One such series of experiments, reported in [5], was carried out with a diploid strain of yeast called 211*B. It is a petite mutant lacking mitochondrial DNA, which makes the labeling of nucleus DNA efficient and specific. Normal brewer yeast can have up to 20% mitochondrial DNA. This would seriously hamper experiments designed to count DSB breaks in the nuclear DNA itself.

The cells were radioactively labeled and irradiated. For each radiation dose a number of aliquots were taken and processed in non growth medium for various lengths of time, extending from zero to 72 hours. Then the cells were lysed, the DNA separated from the protein and sedimentated on a sucrose gradient. For each fraction the amount of radioactivity was recorded. Presumably each fraction corresponds to a certain length (or molecular weight) of double-stranded DNA and the procedure gives a profile of the distribution of fragments lengths. This was also carried out for intact, non-irradiated, DNA, obtaining a profile of lengths of normal chromosomes (17 pairs of various length). To obtain an estimate of the number of DSB's this normal profile was processed through a Monte-Carlo simulation of breakage, with random distribution of breaks (Poissonwise). It can be seen in [4], page 266 that the Monte-Carlo procedure does reproduce the experimental profile with a very good fit except at high doses. For these the experimental data has a proportional surplus of short fragments. (I have been told by Dr. Frankenberg-Schwager that the data in [4] under anoxic conditions should be viewed with caution. The experimenters were unable to reproduce them, for unknown reasons. The data after oxygen absorption are all right).

The results of these particular experiments are recorded in the following graph.

Here insert fig 1

Some salient features are as follows:

1) Immediately after irradiation the average number of DSB's is essentially proportional to dose.

2) After 3 hours in nongrowth medium, the remaining average number of DSB's can be fitted by a linear-quadratic curve. That is, if that average number is N, it can be fitted by $N = ax + bx^2$, where x is the dose and both a and b are positive.

3) After 24 or 48 or 72 hours in non-growth holding the average number of DSB's can be fitted by pure quadratics: $N = cx^2$ where c depends on the holding time.

In a subsequent paper [8] the authors take the trouble to refit the numbers of DSB's immediately after irradiation by a linear-quadratic expression and conclude that

the quadratic term is indeed very small and statistically insignificant.

The decrease in DSB's from 48 to 72 hours is small. One can consider that what remains at 72 hours is not repairable under the non-growth conditions. It could perhaps be partially repairable under growth conditions. For a discussion of this point see [5], [8].

From these features one can immediately obtain some conclusions:

1) The linearity at time zero after irradiation is not compatible with the Chadwick-Leenhouts model. Nor is it compatible with a brutal application of the Kellerer-Rossi dual action theory.

2) The shape of the dose response curve at 3 hours is not compatible with Tobias's repair-misrepair theory. That theory would have lesions been repaired a misrepaired according to a formula of the type $x - bx^2$ where the interaction term gives a *negative* coefficient to x^2.

Another conclusion that can be derived from [5], but not from the graph reproduced above, is that the "saturation" of repair postulated by Goodhead [9] is not present in a significant manner. The authors of [5] carried out separate split dose experiments that indicate that the repair mechanism was still fully efficient at all times and doses. For a description of a similar conclusion, but at lower doses, see [7].

It is true that one cannot completely disregard Tobias's misrepair theory. That it is present and can lead to oversize pieces, larger than the original chromosomes, is documented in[6] page 714, where the sedimentation profile has acquired an "overhang" of large fragments. It is not clear that it could lead to small irreparable pieces accounting for a positive coefficient for x^2.

The effects of repair during the irradiation period can probably be neglected. The irradiation was performed at a dose rate of 130 Gy per minute, thus allowing at most 19 to 20 minutes of irradiation time for the highest dose use (2400Gy). This is enough time for plenty of repair for *single* strand breaks but not for DSB's.

In view of these considerations we attempted to fit the observed results through a modification of the Yang-Swenberg model.

4. A modification of the Yang-Swenberg model

The Yang-Swenberg model specifies that particles impinge on the nucleus of the cell according to a Poisson process in time and space. For low LET radiation, which is the kind considered here for 30 Mev electrons, each primary particle generates a random number of "spurs" acting independently of each other. Each spur has a diameter of about 3 nanometers, comparable to the diameter of a double strand of DNA. Each spur has a probability π_1 of generating a *potentially lethal* lesion and π_2 of

generating a *lethal lesion* with $0 < \pi_1 + \pi_2 < 1$. The values of π_1 and π_2 may depend on the time of arrival of the particle, for instance through the accumulated dose up to that time.

Potentially lethal lesions are those that will become lethal if left unrepaired (or suitably misrepaired). Lethal lesions are those that cannot be repaired or can only be misrepaired, leading to a sure eventual inactivation of the cell.

One can contemplate several variations on this theme, the simplest one being that instead of two, there are three different kinds of lesions that that can be created by a spur: An easy to repair lesion, created with probability π_1, a hard to repair lesion, created with probability π_2 and an irreparable, lethal, lesion with probability π_3. The sum $\pi_1 + \pi_2 + \pi_3$ is still restricted so that $0 < \pi_1 + \pi_2 + \pi_3 < 1$ and the various π_i are still allowed to depend on the previously accumulated dose.

This is not entirely idle speculation, even though it flies in the face of commonly accepted assumptions. One of the commonly accepted assumption is that all DSB's are created equal, owing to the chemistry involved. This, however, is not sufficient grounds to reject our assumption. It has been shown by Bryant [1] that chemically induced breaks of staggered type are much easier to repair than blunt breaks. Also natural DNA is a very complex molecule that presents very fragile and considerably more study parts, because of its complex winding on a protein structure. If a DSB occurs in a well protected part of a nucleosome, it may behave differently from a DSB in a stringy part of the DNA between nucleosomes.

Also the very complex enzymatic machine that performs repairs may be more or less efficient depending on the ease of access of the perceived break. In addition some breaks may lead to DNA unwinding with behavior similar to that of a severed lizard tail while some others remain properly attached to the substructure of histone proteins.

Thus we can contemplate a theory that allows for breaks of different severity. Tobias, among others, has speculated that the really dangerous breaks are chromatin breaks in which the DNA together with the protein substrate are broken. Indeed, attempts to fit his repair-misrepair model to experimental data suggest that the number of so-called "uncommitted lesions" must be rather small, probably one order of magnitude less than the number of DSB's.

Yang and Swenberg assume that for a given rate of irradiation the probabilities π_i are a function of time. To put it differently, they are functions of the accumulated dose at the time a particle impinges on the nucleus. This would need more elaborate justification, but will be retained here for simplicity.

According to this, we shall have three kinds of lesions with different rates of repair, one kind being repaired fast, one slower and one being irreparable. For first

order repair kinetics (no interaction) this would lead to formulas of the following type.

Let Y be the *proportion* of DSB's unrepaired at time t after irradiation. Then Y would have an expectation y = EY of the type

$$y = A(x)e^{-\alpha t} + B(x)e^{-\beta t} + C(x)$$

where A, B and C are positive coefficients that add up to unity, since Y is the *proportion* of DSB's remaining at time t among the total created during the irradiation period. The coefficients A, B and C can depend on the total dose x. In the Yang-Swenberg model they depend on the distribution of the number of spurs generated by one primary particle. For 30 Mev electrons this can be taken zero or one. That is, either the particle does not do anything, or it produces one spur and then escapes from the cell layer. With this assumption on the spurs the exact form of A, B and C as function of x can be obtained from the form of dependence assumed on the probabilities π_i.

Assumptions of the general type as those described in [15], page 57-58, yield cumbersome formulas. The Frankenberg-Schwager experiments of [5] suggest that in the range from 0 to 2400 Grays, with $\alpha > \beta$, A is well approximated by a linear affine function of x while B and C are approximable by strictly linear functions, proportional to x. This of course can only be approximate and for a restricted range since linear or linear affine functions with nonzero coefficients will get out of the permitted $[0,1]$ range for large enough values of x. With such a choice for A and B the relation $y = A(x)e^{-\alpha t} + B(x)e^{-\beta t} + C(x)$ remains linear affine in x for all values of t.

Approximate values, read directly from the Frankenberg-Schwager graph, would be as follows: For the average proportion of remaining DSB's at 3 hours, with dose x in units of 300 Grays, y = .28 + .047x. At 24 hours, y = .045x. At 48 hours, y = .023x and at 72 hours y = .022x. As indicated, these values were read from the regression curves printed on the graph Fig. 7 of [5]. This might not be the best procedure because of distortions in printing, reproducing or errors in reading. However, Dr. Frankenberg-Schwager was kind enough to communicate to us the original data used to make Fig. 7 of [5]. We tried different methods of fitting, with results that differ somewhat from the above, but not enough to change our main conclusion which is that the mixed exponential formula does not fit well. This was rather unexpected, since, even though the bio-physical arguments underlying the derivation of the formula may be entirely fallacious, the formula itself possesses a great deal of flexibility. Indeed, if one permits the range of the coefficients A, B and C to include small negative values and values larger than unity, one can get a most excellent fit.

The fact that, with restricted ranges for A, B and C a fit will be difficult can be seen as follows. For irradiation by 300 Gy's the proportion of DSB's drop from 1.00 to about .3 in just three hours. This forces the coefficient α to be relatively large.

Then the contribution of $A(x) e^{-\alpha t}$ is essentially negligible at $t = 24$ hours or later since $\exp\{-24\alpha\} = [\exp\{-3\alpha\}]^8$.

The remaining terms $B(x) e^{-\beta t} + C(x)$ are then called upon to reproduce the experimental results at 24 to 72 hours. If one take $B(x) = bx$ and $C(x) = cx$ then A has the form $A(x) = 1 - (b + c)x$. This must be in $[0,1]$ for the experimental range, yielding an upper bound of the order of .1 for $(b + c)$ if x is in units of 300 Gy's. Similarly c must be very small. Now the difference

$$B(x) e^{-\beta t_1} + C(x) - B(x) e^{-\beta t_2} + C(x) = B(x) [e^{-\beta t_1} - e^{-2\beta t_1}]$$

for $t_1 = 24$ hours and $t_2 = 48$ hours.

Taking $B(x) = bx$ as above and writing $\gamma = e^{-24\beta}$ one can obtain bounds for γ. Taking .1 as upper bound for b one sees that γ must be of the order of .31. This would imply that, at $t_0 = 3$hours, the term $B(x) e^{-\beta t_0}$ would contribute approximately $(.1)\gamma^{1/8} = (.086)x$, approximately. Plugging this in the formula $y = A(x) e^{-\alpha t} + B(x) e^{-\beta t} + C(x)$ one concludes that for $t_0 = 3$hours the slope of the linear affine relation should be of the order of .057, approximately.

Now it turns out that, for the experimental data, the slopes at 3hrs and 24hrs are essentially the same, being estimated at .047 and .045 here. The difference between .057 and .047 may seem small, however it is considerable. A visual check of the fit shows that it is not acceptable. Since a visual check may not appear very scientific, we also carried out a proper statistical test using an F-ratio statistics. This is of very dubious justifiability here but, as expected it rejects at $p < .05$.

Our conclusion is therefore that the formula $A(x) e^{-\alpha t} + B(x) e^{-\beta t} + C(x)$ does not fit. The same reasoning that led to that formula could also be repeated to argue for more complex mixtures of exponentials such as $\sum_j A_j(x) \exp\{-\alpha_j t\}$. They could be made to fit better, but not well enough to match the almost identity of slopes at 3 and 24 hours. Thus it seems preferable to seek other explanations.

5. Discussion

Assuming that our modification of the Yang-Swenberg model does not fit well, are there any other possible explanations? One possibility, which is admittedly pure speculation, is suggested by the experimental curves themselves. It is that there are two kinds of repair. One of them is fast and operates immediately after irradiation. The other is much slower and it begins to operate only after some length of time of the order of 24 hours. Such a model can be made to fit the experimental curves very closely, but it has little biological support. One could muster some support for it as follows. The yeast used for [5] is diploid. It has been claimed by Resnick [12] that

some form of repair requires an intact sequence of double stranded DNA in the form of an unbroken homologous chromosome. At the high doses considered here, up to 2400 Gays, the number of DSB's is large, probably of the order of 4 or 5 for a chromosome of medium length. It is therefore not impossible that after the first fast repair mechanism has rejoined many of the DSB's a cooperative form of repair can take place at a different rate.

A very different explanation has been suggested in [8] and in a personal communication from Dr. Frankenberg-Schwager. It is that one should not focus only and exclusively on the DSB's. The normal DNA is wound up in a most complex manner on a structure formed of proteins. They together with the DNA determine the geometrical form of the coiling. On the other side, the very delicate machine that performs repair must get hold of the DNA through the protein structure. If the whole complex arrangement has been disturbed, repair can or must proceed at a reduced rate. This is supported by the experiments carried out for [5] and is detailed in [8]. It could be incorporated in the Yang-Swenberg model at little cost. One would just make the repair rates depend on the accumulated dose.

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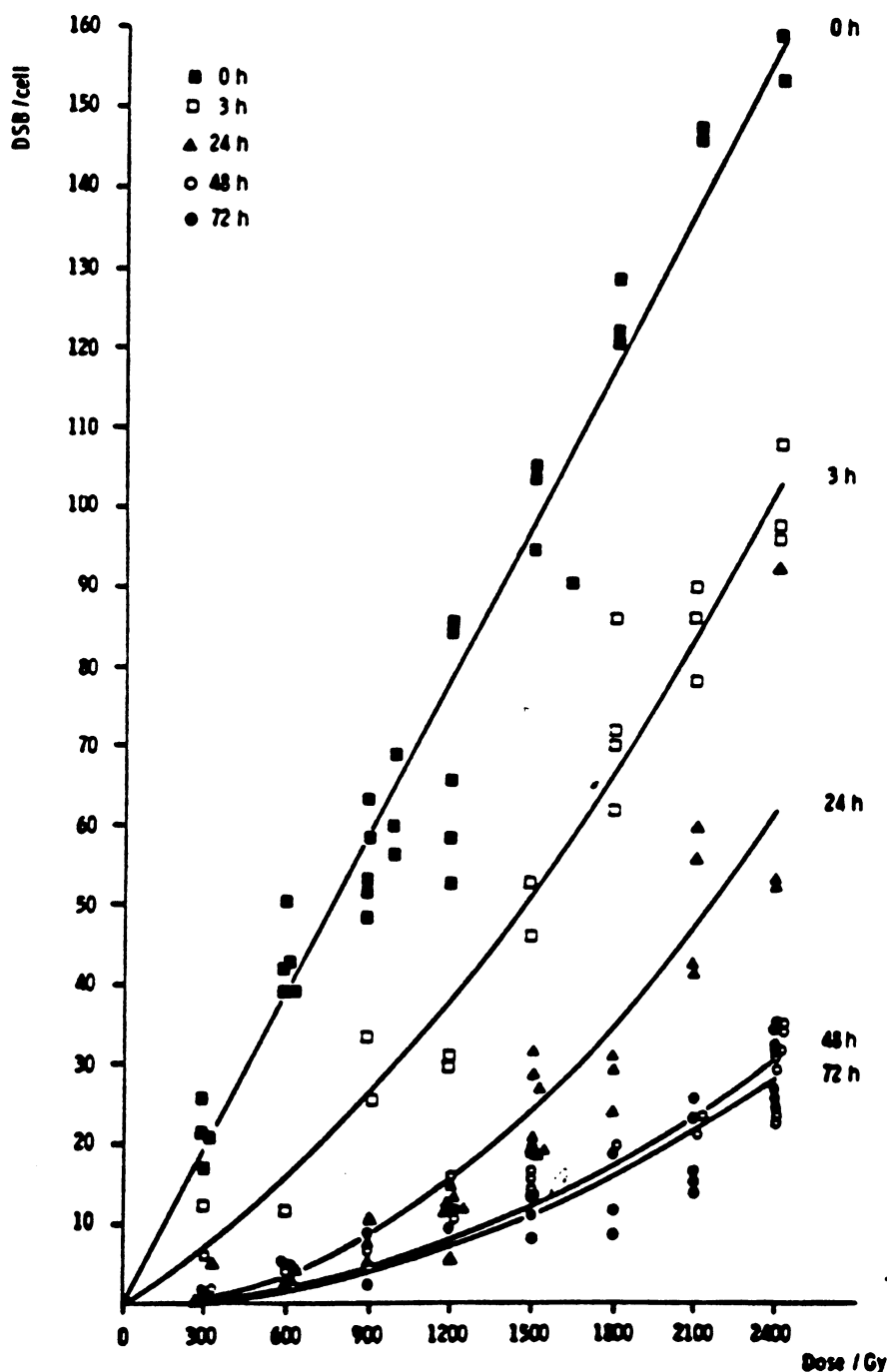
. 7. Repair of dsb under nongrowth conditions. The number of dsb/cell is plotted as a function of Irradiated cells were liquid held for 0 (■), 3 (□), 24 (▲), 48 (O), and 72 hr (●) before analysis ible-strand breakage.

sb repair in irradiated cells also demonstrate that after a 24-hr liquid holding ment not all the reparable dsb are repaired and that cells need longer treatment :pair these dsb. When irradiated cells are plated after 24 hr treatment on ent agar as is the case in survival assays, these remaining reparable dsb may be ired. That this is so has been demonstrated in our preliminary experiments ilts not shown) and in the work of Resnick (32). Our results do, however, ✓ a clear correlation between an increase in colony-forming ability and a

REPAIR OF DNA DOUBLE-STRAND BREAKS