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THE MOLECULAR TARGET THEORY OF CELL SURVIVAL AND ITS APPLICATION IN RADIOBIOLOGY

by

K.H. CHADWICK and H.P. LEENHOUTS

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THE MOLECULAR TARGET THEORY OF CELL SURVIVAL AND ITS APPLICATION IN RADIOBIOLOGY

1. The molecular target theory and the analysis of survival curves
2. The effect of protracted exposure on cell survival
3. The variation in radiation sensitivity in the cell cycle

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ABSTRACT

This report contains the first draft of a theory of radiation induced cell death which has since been rewritten and will be published in the Journal, Physics in Medecine and Biology, under the title « The molecular theory of cell survival ». This initial draft of the theory was written to be general and although it was strongly suspected that the « double-target » used in the theory might be the DNA double helix specific reference to the double helix was avoided. The analysis of radiobiological data indicated that the suspicion was well founded and the theory was rewritten on the assumption that the DNA double helix was indeed the « double-target ». The second version of the theory was found to be more readable and the reader is consequently advised to think specifically in terms of the DNA double helix as the « double-target » when reading this report.

The report is divided into three sections. The first section is concerned with the presentation of a theory for cell survival following radiation which is based on the radiation induced damage to a double molecular target, the double strand break in the DNA double helix. The theoretical expression for cell survival is fitted to experimental results and the implications of the theory with respect to RBE, LET, the oxygen effect and radiological protection are considered briefly.

The second section deals with the effect of protracted exposure on cell survival and it is shown that the theoretical expectations are borne out in the analysis of experimental results. The effect of low dose rate exposure on cell survival is explained on the basis of the repair of broken DNA single strands.

The third section considers the variation of radiation sensitivity with position of the cell in the cell cycle. It is shown that the theory gives a good fit of the survival curve shape in all parts of the cell cycle and that the two coefficients used in the theory have a consistent variation through the cycle for different types of cells. The variations of the coefficients give strong evidence in favour of the assumption that the double molecular target is the DNA double helix and that the possibility for the repair of DNA single strand breaks varies through the cell cycle.

KEYWORDS

SURVIVAL TIME
DNA
MOLECULAR STRUCTURE
RADIATION CHEMISTRY
RADIATION INJURIES
ANIMAL CELLS
RBE
LET
DOSE RATES
BIOLOGICAL REPAIR
RADIOSENSITIVITY
CELL CYCLE

1. THE MOLECULAR TARGET THEORY AND THE ANALYSIS OF SURVIVAL CURVES

Abstract

A theory is presented to explain the effect of radiation on survival. The theory is a modification of the original target theory and contains parameters which take into account the physico-chemical, biochemical and biological effects occurring between the radiation event and the biological result. The theory is presented in two parts; a single-target theory to explain the survival of simple biological units such as enzymes and viruses, and a double-target theory to explain the survival of biological cells. Implications of the theory with respect to RBE, the oxygen effect and radiological protection are mentioned and a fit of the theoretically derived expression to experimental data for 250 kVp X-rays and 15 MeV neutrons is presented.

1. Introduction

The interpretation of radiobiological data on the basis of the target theory of radiation action has been postulated and investigated by Lea (1956) in his book on the Actions of Radiations on Living Cells. Since this proposal much more radiobiological data has been interpreted on the same grounds and the existence of a shoulder on the survival curve of cells has been generally interpreted to imply a multi-target multi-hit effect of radiation.

In 1964, Tym and Todd (1964) proposed an equation to give the surviving fraction S following a dose D which had the form

$$S = e^{-D/D_1}[1 - (1 - e^{-D/D_2})]^n ,$$

where the first term assumes a single event to be responsible for the effect and the second term assumes that n independent events cause the effect.

In 1965, Neary (1965) developed an elaborate theory on the basis of a double target to explain chromosome aberrations and the theory of RBE. In discussing the application of the theory to cell killing Neary suggested that aberrations and killing would not necessarily be directly related but if there were 'double-target sites for cell killing analogous but not necessarily identical with aberration sites' the effect would be similar.

Other authors have proposed models to fit survival curve data (Booz, 1969, Sullivan, 1968) and recently Katz et al (1971) have proposed a model based on the general equation of Tym and Todd and on the delta-ray theory to fit curves for different LET radiation.

Not one of all the models of radiation action on cell survival, which have been presented to date, give a coherent explanation for the variability of radiobiological effect caused by experimental treatments. In this paper a new theory is presented and in following papers attempts will be made to demonstrate how this theory can be used to explain rationally and even quantitatively the various radiobiological effects.

The philosophy which lies behind the theory is as follows:

- it is considered that in the cell there are certain critical molecules, the integrity of which determines the ability of the cell to reproduce,
- the primary action of radiation on the cell is considered to cause molecular disruptions. Any modification of this damage is considered to be a repairing action, or as the chemists would say a 'back' reaction,
- the various radiobiological effects found in a cell type under different irradiation conditions reflect varying degrees of repair,
- these repair processes are considered to embrace the physical recombination processes and energy transfer, the chemical restitution processes and the biochemical enzymatic repair processes.

In order to preserve continuity, certain terminology, which is common in previous radiobiological theories and literature, has been adopted here, for example 'lesion', 'target', 'repair'. These terms are, however, explicitly defined and given a specific meaning in this theory.

An important aspect of this model is that an attempt is made to bridge the gap between the physical measurements of energy absorption and the physico-chemical, biochemical and biological effects which undoubtedly occur following the energy absorption event and which affect the eventual biological result. This is achieved by introducing parameters in the theory which will, through survival curve analysis, acquire a definite biological significance.

2. The molecular target theory

2.1. The single-target theory

Several simple biological entities such as enzymes (Brustad et al, 1966), viruses (Lea 1956) and bacteriophages (Kanazir 1969) appear to have a purely exponential survival curve given by the equation for the target theory of Lea

$$S = e^{-D/D_o}$$

where D_o has often been used to obtain an indication of the size of the target.

The molecular single-target theory is derived in the following way.

a. the mean number of molecular targets damaged per dose per entity is determined
b. some of the damaged molecular targets are allowed to repair
c. a population of biological entities is considered and the mean number of unrepaired damaged molecular targets are used with Poissonian statistics to determine the probability for the effect.

let n_o be the number of critical molecular targets per entity which may lead to inactivation of the entity. The target is thought to be some part of an important molecule, but is not specified.

let k_1 be the probability per target per unit dose that the target is damaged.

and let D be the dose,

then $$\frac{dn}{dD} = -k_1 n \text{ and } n = n_o e^{-k_1 D} ,$$

so that the number of targets damaged per entity is

$$n_o - n = n_o (1 - e^{-k_1 D}).$$

It is now assumed that

r is the proportion of damaged targets which are restituted or repaired per entity

and f = 1-r is the proportion of damaged targets per entity which are not restituted or repaired and can lead to radiation damage.

Thus the mean number of damaged targets per entity available to cause an effect at a dose D is

$$f\, n_o (1 - e^{-k_1 D}) ,$$

then using Poissonian statistics the probability at dose D for biological inactivation by $^1/_p$ damaged targets per entity is

$$E = 1 - e^{-p\, f\, n_o (1 - e^{-k_1 D})} ,$$

where p is a proportionality factor connecting damage and inactivation.

The surviving fraction is given by

$$S = 1 - E = e^{-p\, f\, n_o (1 - e^{-k_1 D})} .$$

The parameter k_1 is connected with a target having molecular dimensions and is thus very small so that the equation can be approximated to

$$S = e^{-p\ f\ n_o\ (1\ -\ (1\ -\ k_1 D))}$$

or

$$S = e^{-p\ f\ n_o k_1 D}$$

This equation is similar in form to the Lea target theory equation but the interpretation of the exponent is essentially different. It is also very similar to the equation of Braams (1963) for the modification of the inactivation of enzymes. The exponent contains not only the target 'cross section' but also the number of targets per entity and the proportion of targets which remain unrestituted and unrepaired.

Thus a basic conclusion from this modified theory is that the irradiation of the biological entities under aerobic and anaerobic conditions will both reveal exponential curves having a different exponent, which is not connected with a change in target size, but with a change in the value of f, the ability with which a damaged target can restitute or repair itself. In other words, the oxygen interferes with the restitution process and in the same way radiation sensitizers and protectors will also affect this value of f.

2.2. The double-target theory

In order to explain the cell survival curves which are normally termed multi-hit curves use is made of the basic molecular single-target theory but an additional assumption is made; namely, that the cell death is caused by a number of lesions, and each lesion

is a result of the combination of, or interaction between two damaged critical targets. It is not essential to the theory that the critical targets are identical, such as the two strands of the DNA helix (Neary 1965) or different, such as the DNA chain and the cell membrane (Alper 1969).

These two molecular targets may be inactivated through two modes of action

a. both targets are hit in one radiation event

b. each target is hit independently by different radiation events.

Consider the second mode of action (b) and let

n_1 be the number of critical targets of type 1 per cell,

n_2 be the number of critical targets of type 2 per cell,

k_1 be the probability per target per unit dose that the type 1 target is damaged,

k_2 be the probability per target per unit dose that the type 2 target is damaged,

f_1 the proportion of damaged type 1 targets not restituted or repaired,

f_2 the proportion of damaged type 2 targets not restituted or repaired,

and if a proportion Δ of the dose D inactivates via the first mode of action and $(1-\Delta)$ is the proportion giving the second mode of action, where Δ will be dependent on the LET of the radiation, then the number of type 1 targets available for the biological effect is

$$f_1 n_1 (1 - e^{-k_1(1 - \Delta)D}) ,$$

and of type 2 targets

$$f_2 n_2 (1 - e^{-k_2(1 - \Delta)D}) .$$

Now, if ε is the proportion of these damaged targets which combine or interact and are available to produce cell death, the mean number of combined damaged targets, i.e. lesions, is

$$\varepsilon f_1 f_2 n_1 n_1 (1 - e^{-k_1(1 - \Delta)D})(1 - e^{-k_2(1 - \Delta)D}) .$$

It seems likely that for two damaged targets to combine to cause a lesion they will need to be associated in space and time and it is this association which is expressed in ε.

Consider now the first mode of action (a) and let

n_o be the number of 'double targets' for this mode of inactivation, where $n_o \leqslant n_1, n_2$,

k_o be the probability per 'double target' per unit dose that the double target is damaged,

then the mean number of damaged 'double targets' or lesions is

$$n_o (1 - e^{-k_o \Delta D}) .$$

Thus the total mean number of lesions per cell after a dose D is

$$n_o(1 - e^{-k_o \Delta D}) + \varepsilon f_1 f_2 n_1 n_2 (1 - e^{-k_1(1 - \Delta)D})(1 - e^{-k_2(1 - \Delta)D}) .$$

If f_o is the proportion of lesions not restituted or repaired, then using Poissonian statistics the probability for cell death per dose D is

$$E = 1 - e^{-pf_o[n_o(1-e^{-k_o \Delta D}) + \varepsilon f_1 f_2 n_1 n_2 (1-e^{-k_1(1-\Delta)D})(1-e^{-k_2(1-\Delta)D})]},$$

where p is a proportionality factor connecting lesions and cell death.

The surviving fraction is given by

$$S = 1 - E$$

and

$$S = e^{-pf_o n_o (1-e^{-k_o \Delta D})} . e^{-pf_o \varepsilon f_1 f_2 n_1 n_2 (1-e^{-k_1 (1-\Delta) D})(1-e^{-k_2 (1-\Delta) D})} .$$

This can be approximated, as before, to

$$S = e^{-pf_o n_o k_o \Delta D} . e^{-pf_o \varepsilon f_1 f_2 n_1 n_2 k_1 k_2 (1-\Delta)^2 D^2} .$$

This equation has exactly the same form as, that given by Kellerer and Rossi (1971) although the starting point is different. It is also the form found by Sinclair (1966) who used a mathematical analysis of some cell survival data to obtain the expression, without having a model on which to base it.

3. Discussion

Certain general statements can now be made in order to gain more insight into the implications of this expression.

The first term will dominate the survival only when

$$pn_o f_o \Delta k_o \gg pf_o \varepsilon f_1 f_2 n_1 n_2 k_1 k_2 (1-\Delta)^2 D .$$

This will occur at low doses in general but will certainly be dependent on Δ or the LET of the radiation. As Δ, or the LET, increases the term dependent on D will play an increasing role. The expression predicts, however, that even for sparsely ionizing radiation the survival will be proportional to dose at very low doses. This has also been predicted by Sullivan (1968), and Rossi (1971) from considerations of the stochastic properties of radiation. This fact is of relevance to radiological protection though it should be borne in mind that the expression only applies to cell survival and not to organ survival when the death of a few cells may have no effect on the function of the organ. In order to see how this

expression can be used for the prediction of genetic changes or cancer induction it will be necessary to compare it with several sets of experimental data.

It seems reasonable to expect that the restitution or repair of a lesion will be, if not impossible, certainly more difficult than the restitution or repair of a single damaged target and that $f_o > f_1$ or f_2. Thus, the effect of the first mode of inactivation will be more difficult to influence than the effect of the second mode of inactivation, especially as the hits in the first mode of inactivation will be associated in time and space by definition. Thus the effect of oxygen in particular, and sensitizers and protectors in general will be dominant in the second mode of inactivation and will be revealed in a change in the value of the product $f_1 f_2$. This implies two things, that the effect of high LET radiation will be less modifiable than that of low LET radiation in general, a common radiobiological finding, but that at very low doses of electromagnetic radiation, where the linear term in dose dominates, the modification of the effect will also be small. This prediction, if verified, is also of importance for radiological protection.

At higher doses the survival will be dominated by the quadratic term in dose and this effect has been discussed in general by Kellerer and Rossi (1971), and by Neary (1965) for the special case of chromosome aberrations. This, plus the fact that the importance of the linear term in dose will increase with increasing LET will lead to the relationship between RBE and X-ray dose found by Kellerer and Rossi (1971).

Figure 1 gives the analysis of the survival curves for T-1g cells for 250 kVp X-rays and 15 MeV neutrons taken from Barendsen (1968). The X-ray curve is detailed and analysis gives the expression .

$$S_x = e^{-2.38 \times 10^{-3} D} \cdot e^{-2.7 \times 10^{-6} D^2}$$

which indicates that the linear term in D is substantial and plays an important role up to approximately 600 rad. The analysis of the 15 MeV neutron curve gives the expression

$$S_n = e^{-7.7 \times 10^{-3} D} \cdot e^{-5.4 \times 10^{-6} D^2}$$

which gives a limiting RBE value for low doses of

$$RBE_{lim} = \frac{7.7 \times 10^{-3}}{2.38 \times 10^{-3}} = 3.2.$$

4. Conclusions

The molecular target theory presented here starts from four basic assumptions:

1. the target has molecular dimensions and target damage is concerned with chemical bond breakage.
2. the damaged target can be restituted or repaired.
3. for the description of survival curves for cells the interaction of two damaged targets is necessary to cause a lesion.
4. a certain number of lesions cause cell death.

In the simplified form it gives the normal exponential survival curve similar to that of Lea, but the 'cross section' involved is no longer a value to be used to identify some structure in the biological entity.

In the double-target form the theory gives an equation for the shape of the survival curve for cells, gives predictions of relevance to radiological protection, some of which have recently been vindicated and contains implicitly in the exponents, factors governing the effect of oxygen, sensitizers and protectors on the survival curve.

The theory is general for all ionizing radiation but contains a factor Δ which will depend on the LET of the radiation. It is reasonable to expect that more detailed mathematical expressions for the parameters f and ε will become available as a result of the analysis of more radiobiological experiments, and that physical measurements will reveal more information on the parameters Δ and k.

The theory makes no predictions about the targets, they may be DNA strands, cell membranes, radicals or other parts of the cell. Further analysis may reveal more information about the effect of oxygen, sensitizers and protectors, the targets themselves, the effect of dose rate and the effect of fractionation.

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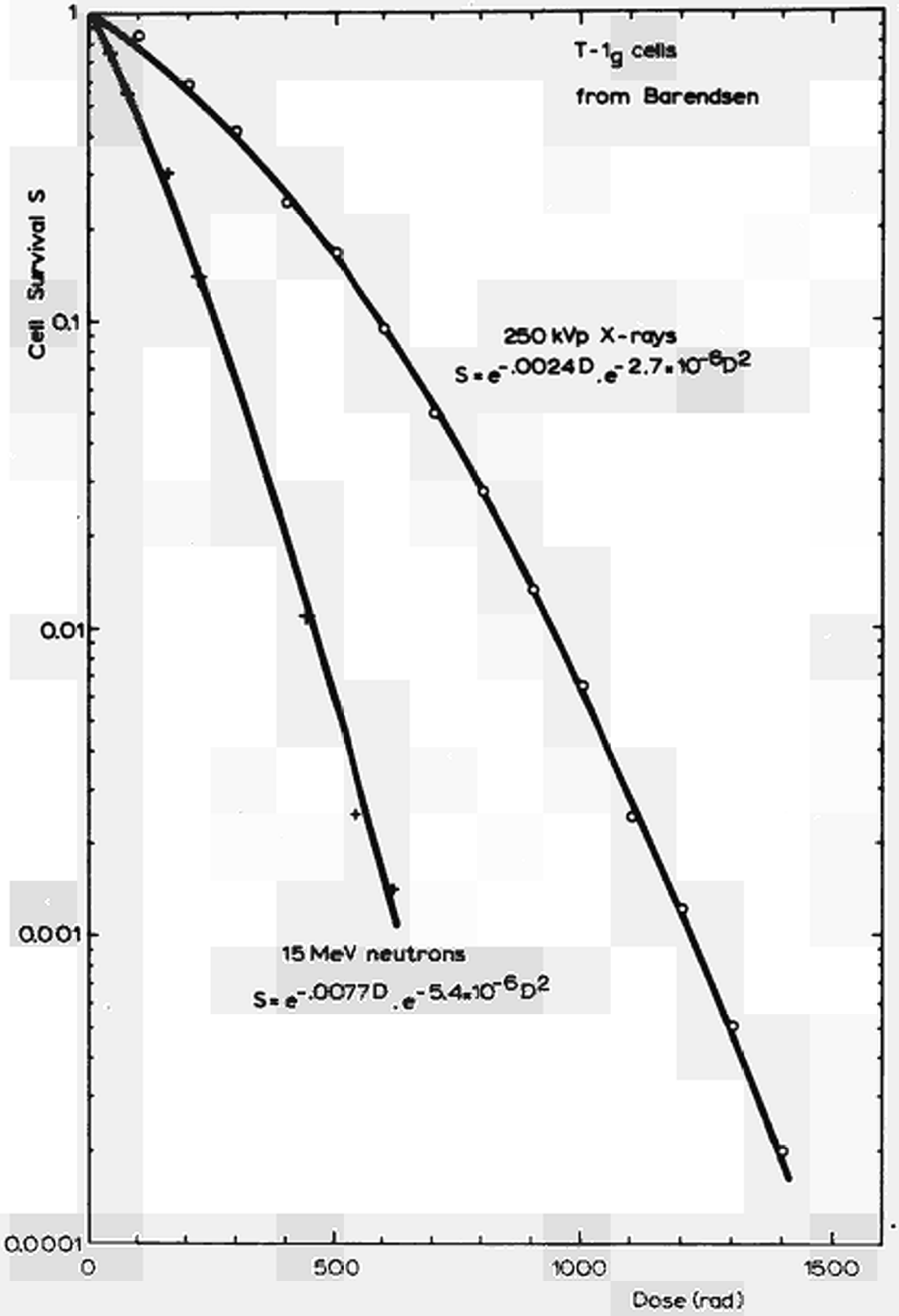

Figure 1. Fit of theory to survival curves obtained for cultured cells of human origin (T-1g cells) irradiated with 15 MeV neutrons and 250 kVp X-rays. (Barendsen 1968).

2. THE EFFECT OF PROTRACTED EXPOSURE ON CELL SURVIVAL

Abstract

The molecular target theory is reviewed briefly and the possible influence of dose rate on the different parameters in the theory is considered. The theory gives an equation for cell survival as

$$S = e^{-aD} \cdot e^{-bD^2}$$

and it is concluded that the coefficient 'a' will be unaffected by dose rate, except possibly at extremely high dose rates, but that the 'b' parameter will be influenced by dose rate. In protracted exposures the influence on 'b' by dose rate is governed by the repair of damaged single molecular bonds during irradiation. Several cell survival curves measured at various low dose rates have been analysed and give results which are consistent with the theory. The possible connection between the metabolically controlled repair of the damaged single molecular bond and the repair of DNA single strand breaks following irradiation is mentioned.

1. Introduction

The molecular target theory for the analysis of cell survival data has been presented in a previous paper (Chadwick and Leenhouts 1972). Essentially, the theory gives an expression for cell survival following radiation as

$$S = e^{-aD} \cdot e^{-bD^2}$$

and is based on the idea that there are in a cell many critical molecular targets which can be damaged by radiation, that the targets may be repaired, that for cell death two damaged targets must combine to give a lesion and that a number of lesions induce cell death. The first term in the expression (e^{-aD}) gives the chance that two critical targets are hit in one radiation event and $a = p\, f_o\, n_o\, k_o\, \Delta$, contains parameters describing the number and cross-section of such a 'target' (n_o, k_o), the chance of repair (r_o, $f_o = 1 - r_o$), the relationship between the number of lesions and cell death (p) and the fraction of dose going into this mode of damage (Δ). The second term (e^{-bD^2}) gives the chance that two independently hit and unrepaired targets will combine to give a lesion and $b = p\, f_o\, \varepsilon\, f_1\, f_2\, n_1\, n_2\, k_1\, k_2\, (1-\Delta)^2$ contains parameters describing the numbers and cross-sections of the two targets (n_1, n_2, k_1, k_2), the possible repair of the targets before combination to form a lesion (r_1, r_2; $f_1 = 1 - r_1$, $f_2 = 1 - r_2$), the chance that they will combine to form a lesion (ε) and the fraction of dose going into this mode of damage ($1-\Delta$).

The effect of low dose rate exposure on cell survival has been previously explained using single target multi-hit kinetics and 'heavy' and 'light' hits (Porter 1965) on the basis of the repair

of sub-lethal damage. However the values of D_o and extrapolation number (n) obtained from the analysis do not have any biological significance and the sub-lethal damage is also not specified. (Fox and Nias 1970).

This paper is concerned with the application of the molecular target theory to the effect of low dose rate exposure in radiobiology and presents the results of this analysis. The biological significance of this interpretation is discussed.

2. Analysis

The optimal values of the coefficients 'a' and 'b' have been determined from experimental cell survival data using the χ^2 method.

The value of $q^2 = \sum_{i=1}^{n} \left(\frac{T_i - E_i}{\Delta E_i}\right)^2$ is minimalised

where T_1 is the theoretical value of the logarithm of the surviving fraction for dose D_i

E_i is the corresponding experimental value

ΔE_i is the error in E_i

and n is the number of experimental points.

Then $q^2 = \sum_{i=1}^{n} \left(\frac{-aD_i - bD_i^2 - E_i}{\Delta E_i}\right)^2$ and in the minimum

$\frac{dq^2}{da}$ and $\frac{dq^2}{db} = 0$. The simultaneous equations thus obtained can be solved to give 'a' and 'b'. The minimum value of q^2 is called χ^2.

The errors in the values of 'a' and 'b' have been evaluated by varying 'a' and 'b' until $q^2 = \chi^2 + 1$, (Emé 1966).

In experimental results which give an indication of experimental errors, these have been used to determine ΔE_i. In cases where no indication of experimental error has been given the value of ΔE_i has been estimated, usually at a 10 % level. In the situation when the value of χ^2 exceeds the degree of freedom (n - 2) the errors ΔE_i have been readjusted to make $\chi^2 \simeq n - 2$, (Wapstra, Nigh and v. Lieshout 1959). The error in the value of dose has been neglected. The accuracy with which the values of 'a' and 'b' can be determined depends to a large extent on the accuracy and completeness of the radiobiological data. The detailed curve of Barendsen (1968) for T - 1g cells with 250 kVp X-rays, for example, gives values of 'a' and 'b' (Chadwick and Leenhouts 1972) at a 5 % accuracy level. However, this is not always the case and it is important to have good radiobiological data and a complete survival curve in order to be able to make a useful analysis.

3. The dose rate effect - considerations using the molecular target theory

Dose rate involves two essential parameters each of which may have a specific role, one parameter is time and the other is the concentration of radiation products per unit time interval.

In considering the dose rate effect from the point of view of the molecular target theory it is convenient to take each coefficient 'a' and 'b' separately.

In the coefficient $a = p f_o n_o k_o \Delta$

- the parameters p, n_o and Δ are independent of time and concentration of products
- the parameter k_o will be dependent upon the geometrical relationship between the two molecular targets and may be altered by cell

proliferation. However, in an asynchronous cell population k_o will be a mean value and is not expected to change drastically with time.

- the parameter f_o concerns the repair of a lesion and this repair will certainly need time, but unless the repair process is influenced by the radiation, which is doubtful, or unless it is affected by the concentration of radiation products, which may occur at extremely high dose rates, the process will remain constant throughout and after the irradiation, and will not be dependent on dose rate.

Consequently the coefficient 'a' is not expected to be affected by low dose rate variations.

In the coefficient $b = p\, f_o\, \varepsilon\, f_1 f_2 n_1 n_2 k_1 k_2\, (1 - \Delta)^2$

- the parameters p, f_o, n_1, n_2, k_1, k_2, and $(1 - \Delta)$ are not expected to be dependent on time or concentration of radiation products.
- the parameters f_1 and f_2 are concerned with the restitution and repair of a single damaged target before combination with a second damaged target to produce a lesion. The single damaged target is considered to be a broken molecular bond and thus restitution and repair of this effect may occur, starting from the ionisation event, by physical, chemical, biochemical and biological processes. Each of these processes will have one, or a series of time constants associated with it and may also be dependent on the concentration of electrons, ions or other radiation products in the neighbourhood of the broken bond. If just one process of repair is considered then with one broken bond the chance that the correlated second target is damaged before the first bond is repaired will be dose rate dependent. Thus, any radiation treatment given in a time

period which is in the neighbourhood of one of the time constants of the repair and restitution processes will manifest a dose-rate effect. Equally well will be the fact that treatments given in time periods differing significantly from the repair time constants will not show a significant dose rate effect.

The parameter ε governs the chance that two single damaged targets will combine to produce a lesion. There are two possible considerations involved; the first is that the damaged targets are immobile and that ε expresses the probability that a second target which is correlated with the first damaged target is also damaged. In this case ε will not be dependent on the dose rate although the effect will, due to the repair time of the first damaged target. The second consideration is that the damaged targets are mobile and can diffuse to react with each other. In this case ε will be concerned with diffusion rates and distances between damaged targets and will thus be dependent on time and concentration of the damaged targets.

Consequently, the coefficient 'b' is expected to be dose rate dependent in certain ranges of dose rate and an analysis of dose-rate effect curves for cell survival should give little change in the 'a' coefficient and an important change in the 'b' coefficient.

The theory predicts that as the radiation quality increases the value of Δ will increase (Chadwick and Leenhouts 1972) and more of the radiation effect will occur via de double-target-hit-in-one-event process. Thus with increasing radiation quality a decreasing dose rate effect will be expected, and in this respect the theory is in agreement with the considerations of Barendsen (1968).

4. Results

The analysis of survival data using the molecular target theory has been applied to various experimental results on dose-rate effects. The results are presented in Figures 1 - 6, where the curves have been calculated using the best values of 'a' and 'b'. The values of 'a' and 'b' with errors are given in Table 1.

5. Discussion

The results of the analysis, whilst being in general agreement with theory in giving no significant change in the 'a' value, are a little disappointing as some of the coefficients have large errors. The dose-rate effects considered here concern protracted exposures with proliferating cell populations and Fox and Nias (1970) have indicated the problems which cell proliferation will cause to any analysis of such results. In this case, the irradiation history of the cell population is altered during irradiation and the new cells have received less than the total dose. This effect will make the analysis less accurate, and will result in a changing value of the 'b' coefficient during irradiation and therefore along the curve.

Fox and Nias (1970) have taken the effect of cell proliferation into account and have avoided it by using a stationary cell population for both the short and protracted irradiations. This experiment gives a very good result on analysis; the 'a' value remains the same, the 'b' value is radically altered and the errors in all values are small.

The experiments of Hall and Bedford (Bedford and Hall 1963, Hall and Bedford 1964), and Berry (1962, 1968) while giving inaccurate values of the parameters 'a' and 'b' are in general accordance with the theory. The 'a' values remain relatively

constant and the 'b' values show an increase with increasing dose rate.

These protracted exposures are almost certainly concerned with the biological repair processes and involve irradiation times of the same order of magnitude as those found necessary in fractionation experiments to allow for the repair of damage between fractions. In these protracted exposures the 'b' values tend to, or are, zero, indicating that the repair of single damaged targets is complete and that the only damage occurs through the 'double-target-hit-in-one-event' effect. In this respect it is interesting to note that in cells irradiated at low temperature ($-196^{\circ}C$) neither dose rate nor fractionation effects have been found (Nias and Ebert 1969); at this low temperature the cell proliferation problem was removed and also the metabolic processes were stopped. Fox and Nias (1970) avoided the cell proliferation problem but still found a dose rate effect, which the theory indicates to be concerned with the repair of damaged single molecular targets. Pohlit (1968) has found that the recovery following a split dose fractionation is concerned with a metabolic process. Thus it seems that the low dose rate effect can be connected with a metabolically controlled repair of damaged single molecular bonds via the theory.

The repair of radiation induced single strand breaks in DNA in cells has been noted after irradiation (Lett et al 1967, Lohman 1968, Humphrey et al 1968, Painter 1970) and also unscheduled DNA synthesis has been found in different phases of the cell cycle apart from the S phase also following irradiation (Painter 1970, Painter and Cleaver 1967). On this basis it is tempting to link the metabolically controlled repair of a damaged single molecular

bond, which is quantitatively related to the low dose-rate effect by the theory, to the metabolic process of repair of DNA single strand breaks. It is then equally tempting to assume that the double target used in the theory is in fact the DNA double helix, but it must be concluded that the evidence for the basis of this assumption is at present circumstantial.

6. Conclusion

It is concluded that the dose-rate effects found in cell survival can be expected on the basis of the molecular target theory and that the analysis of experimental results are in support of the theoretical considerations.

The low dose rate effect seems to be compatible with the biological repair of a single damaged molecular target and circumstantial evidence indicates that a connection may exist between the repair of this broken molecular bond and the repair of single strand breaks in DNA.

The theory predicts that the dose rate effect for densely ionizing radiation will be much less obvious than for sparsely ionizing radiation.

Acknowledgements

The authors wish to acknowledge the critical discussion which they have had with Prof. R. Braams (Radiation Biophysics Group, Utrecht University) and our colleagues in the institute, especially Dr. K.J. Puite and Drs. W.F. Oosterheert.

Cell type and irradiation	Dose rate	a x 10^3 (rad^{-1})	b x 10^6 (rad^{-2})	Ref.
Chinese hamster cells in vitro	100 rad/min	1.6 ± 0.2	2.8 ± 0.4	Fox 1970
	25 rad/h	1.6 ± 0.2	0 ± 0.1	
Hela cells in vitro	44.9 rad/min	3.8 ± 0.3	1.3 ± 0.3	Hall 1964
	16.9 rad/min	3.6 ± 0.2	1.0 ± 0.4	
	2.37 rad/min	3.7 ± 0.2	0 ± 0.3	
in vitro	19 rad/hr	3.0 ± 0.5	1.1 ± 0.9	Hall 1963
	9.5 rad/hr	3.1 ± 1.0	0 ± 3	
P 388 lymphoma cells in vitro	110-130 rad/min	5.1 ± 1.0	1.8 ± 1.3	Berry 1968
	40 rad/h	3.1 ± 2.3	0 ± 3	
in vivo	110-130 rad/min	2.0 ± 0.5	0.28±0.22	Berry 1968
	40 rad/h	1.3 ± 0.3	0.085±0.085	
	20 rad/h	1.4 ± 0.2	0 ± 0.1	1962

Table I: Results of the analysis of several cell survival curves measured at different dose rates.

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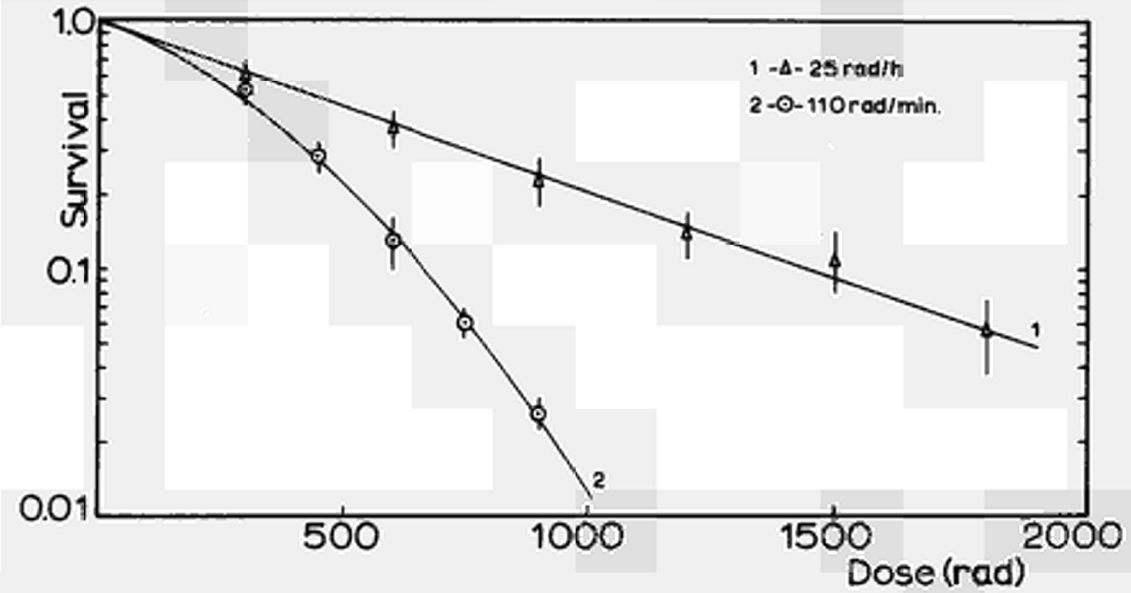

Figure 1 Analysis of Chinese hamster cell survival data from Fox and Nias (1970).

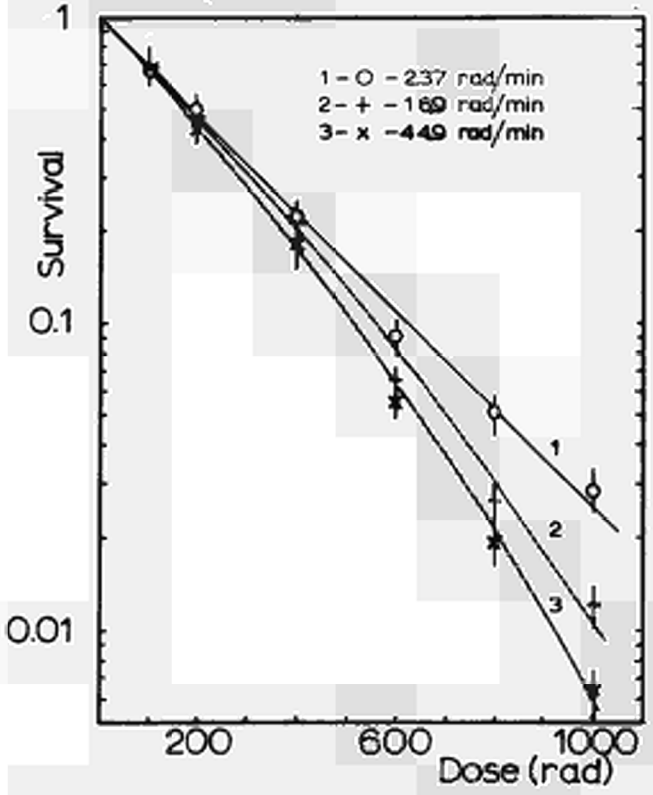

Figure 2 Analysis of Hela cell survival data from Hall and Bedford (1964).

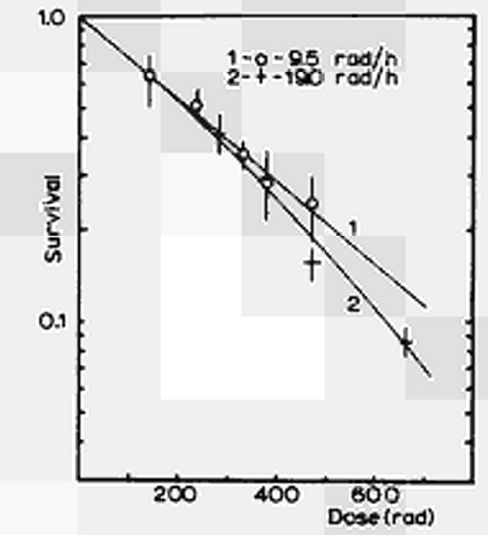

Figure 3 Analysis of Hela cell survival data from Bedford and Hall (1963).

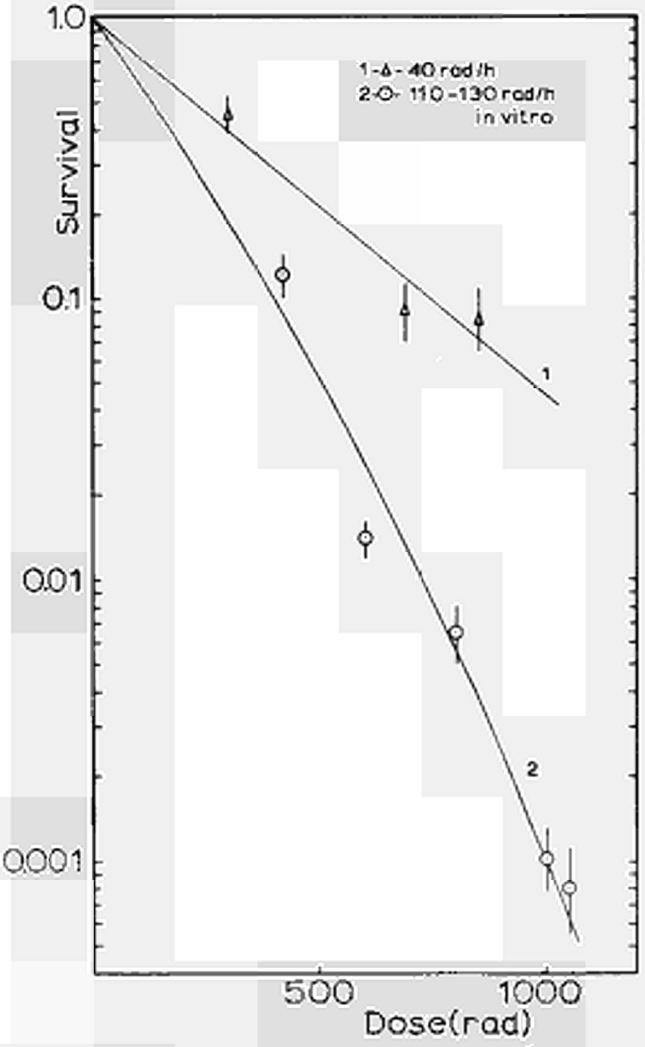

Figure 4 Analysis of P-388 ascitic leukemia cell survival data from Berry (1968) (in vitro).

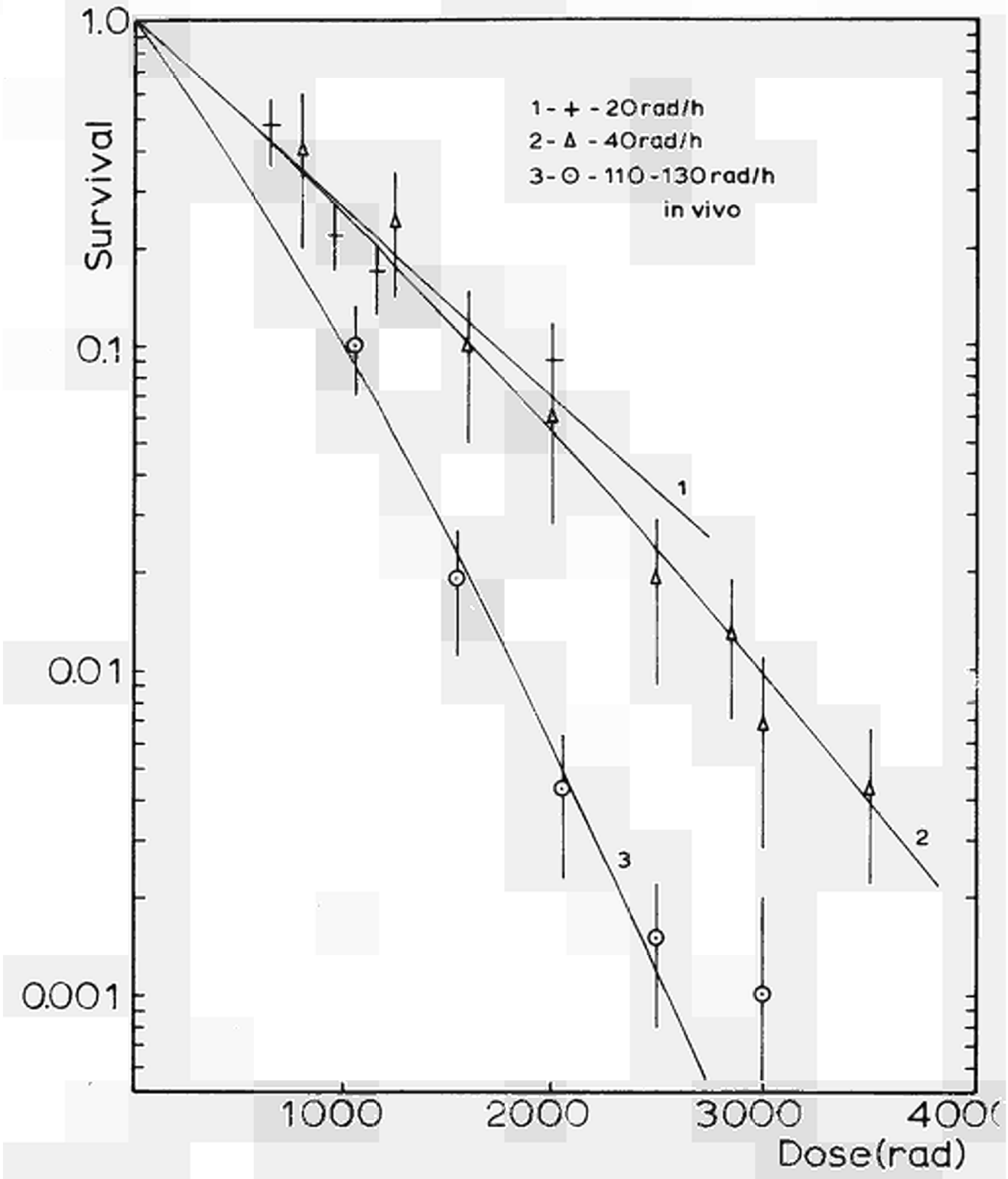

Figure 5 Analysis of P-388 ascitic leukemia cell survival data from Berry (1968) and Berry and Cohen (1962) (in vivo).

3. THE VARIATION IN RADIATION SENSITIVITY IN THE CELL CYCLE

Abstract

The molecular target theory is used to analyse the variation of radiation sensitivity in the cell cycle. It is shown that the equation for cell survival derived in the theory

$$S = e^{-aD} \; e^{-bD^2}$$

can be used to fit the varying shapes of survival curve found in synchronized cell populations in different stages of the cell cycle. Further analysis of many experimental results reveals that the coefficients 'a' and 'b' exhibit a consistent variation through the cell cycle. Consideration of the variation of 'a' with the molecular biology of the cell leads to the conclusion that the critical lesion leading to cell death is a double strand break in the DNA double helix.

1. Introduction

The variation in the radiation sensitivity of cells with the position in the cell cycle is a well known phenomenon. Many experiments have been carried out using synchronized cell cultures in vitro (Terasima and Tolmach 1963, Sinclair and Morton 1966, Vos et al 1966, Sinclair 1969, Thompson and Humphrey 1969) and recently a review has been made by Sinclair (1968). The situation is still somewhat confused and whilst some general statements can be made about the sensitivity during the cell cycle, there are also important differences between different cell types. Sinclair has considered, in his review, three different cell types, Chinese hamster lung cells of the V79 line, HeLa cells and mouse L cells. He summarizes the principle features of the X-ray survival-age response as follows:

1. Cells are generally most sensitive at mitosis.
2. If G_1 is of appreciable length, a resistant period is usually evident early in G_1, followed by a decline in survival towards S. The end of G_1 may be as sensitive as mitosis.
3. In most cell lines, resistance rises during S to a maximum in the latter part of S. This is usually the most resistant part of the cycle.
4. In most cell lines the G_2 period is sensitive, perhaps as sensitive as in mitosis.

Sinclair goes on to point out that the most unsatisfactory feature of the analysis of studies of synchronized cell populations results, because the changing shape of the survival curve through the cell cycle cannot be coherently interpreted using any variation of the hit and target models of cell survival. He also states that

at some stages in the cell cycle the shape of the survival curve is such that it cannot be adequately represented by any choice of multi-target single-hit relationships.

The molecular target theory (Chadwick and Leenhouts 1972) offers a different approach to the analysis of survival curves than that used in the traditional hit and target theories. Therefore an attempt has been made to analyse the variation of radiation sensitivity in the cell cycle using this theory. This paper presents the results of this analysis and discusses their implications.

2. Analysis

The molecular target theory gives a relationship for cell survival as

$$S = e^{-aD} \cdot e^{-bD^2}$$

where a is the chance that a double target is damaged in one radiation event and

b is the chance that two targets are damaged in seperate radiation events. The complete theory and the component parameters of the coefficients 'a' and 'b' have been discussed previously (Chadwick + Leenhouts 1972).

Various groups of data on synchronous cell survival have been analysed using the molecular target theory and the parameters 'a' and 'b' have been determined. The data which have been analysed have been chosen mostly because they give several measurements of the survival curve through the cell cycle. In several publications colony survival is plotted instead of cell survival because in these cases the synchronization technique causes some cell multiplicity. The colony survival has been recalculated to single-cell-

survival using the equation given by Sinclair and Morton (1966) and the values of cell multiplicity given in the relevant publications. Sinclar and Morton have shown in one example that the recalculated cell survival fits the measured single-cell-survival quite well and these two curves have been analysed to see if the parameters 'a' and 'b' were significantly different. The parameters 'a' and 'b' were in fact different (see Table I) but the difference is not significant.

	Recalculated curve using cell multiplicity $\bar{N} = 2.0$	Measured curve
'a' coefficient (rad^{-1})	$(11.0 \pm 3.0) \times 10^{-4}$	$(5.8 \pm 3.5) \times 10^{-4}$
'b' coefficient (rad^{-2})	$(1.6 \pm 0.3) \times 10^{-6}$	$(2.3 \pm 0.5) \times 10^{-6}$

Table I. Comparison of coefficients estimated from a single-cell-survival curve and the corresponding curve recalculated from the colony survival curve. Data from Sinclair and Morton (1966).

The results recalculated from colony survival are consequently considered to be accurate enough to indicate the trend in the coefficients 'a' and 'b' through the cell cycle.

Figure 1 demonstrates the fitting of the molecular target theory to the survival curves measured at different parts of the cell cycle. The data have been taken from Sinclair (1969).

Figure 2 gives the variation of the coefficients 'a' and 'b' through the cell cycle for a series of different measurements taken from references (Sinclair and Morton 1966, Kruuv and Sinclair 1968,

Sinclair 1969, Dewey et al 1970, Dewey et al 1971, Vos et al 1966). (see Table II). The standard errors estimated in 'a' and 'b' are also indicated.

3. Discussion

Figure 1 shows as an example that the molecular target theory can be used to fit the changing shape of the survival curve throughout the cell cycle giving an analysis based on the variation of two coefficients through the cell cycle.

Figure 2 shows that the variation of the coefficient 'a' throughout the cell cycle is consistent in the cell data which have been analysed. The coefficient 'a' is smallest in the middle of the S phase and is highest in Mitosis and the beginning of the G_1 phase. The coefficient 'b' also demonstrates a variation throughout the cell cycle which is similar for all the data which have been analysed. The variation is different from that of the 'a' coefficient and in general 'b' has a peak in the late G_1 or early S phase.

It would appear from these results that the apparently unsystematic behaviour of different cell lines is reflected in the various combinations and relative magnitudes of the two coefficients 'a' and 'b'. For instance, Figure 2 does not indicate any significant difference between cells having a long or short G_1 phase as has been previously noted in the literature (Sinclair 1969) and the minimum sensitivity often found at the end of the S phase can be attributed to the low but increasing 'a' value coupled to the low but decreasing 'b' value.

It is interesting to consider the coefficients and the variation of the coefficients through the cell cycle in more detail with reference to the biochemistry and metabolism of the cell.

The coefficient 'a' has a minimum in the middle of DNA synthesis and replication and it seems to be reasonable to try to relate the double target for cell inactivation to the DNA double helix and to see if any of the component parameters in 'a' can be expected to vary as a result of DNA synthesis and replication. There have been several publications recently indicating a connection between DNA damage and cellular inactivation (Elkind and Painter 1971, Szybalski 1966, McGrath and Williams 1966, Drasil etal al 1971, Singh and Gopal-Ayengar 1971, James et al 1971). In a previous paper concerned with the effect of protracted exposure on cell survival a connection was made between the repair of a damaged single molecular bond and the repair of single strand breaks in DNA (Leenhouts and Chadwick 1972).

The coefficient $a = pf_o n_o k_o \Delta$ where

- p relates the number of lesions per cell to cell inactivation (a lesion is considered to be caused by a damaged double target)
- f_o is related to the chance that a damaged double target can be repaired
- n_o is the number of double targets
- k_o is the 'cross section' of a double target

and Δ is the fraction of dose going into this mode of inactivation.

- the parameter n_o, assuming that it is related to the DNA content of a cell, increases as the cell cycle progresses and doubles during the DNA synthesis. However a double strand break in the DNA before replication will be reproduced in the replication process and will affect each of the two new cells; a double strand break in one

of the two DNA double helixes formed after replication will only affect one of the two new cells formed on mitosis. Thus the change in n_o is compensated by the replication process and this is expressed mathematically by allowing the product pn_o to remain constant throughout the cell cycle.

- the parameter f_o, related to the repair of a double strand break in DNA, may vary throughout the cell cycle, although the repair of a double strand break in DNA has as yet only been demonstrated in microorganisms (Burrell et al 1971). However, the parameter f_o is also common to the coefficient 'b' and thus if f_o is the dominating factor the coefficients would be expected to show a similar variation through the cell cycle.

- the parameters k_o and Δ are interrelated; k_o represents the 'cross-section' of the double DNA strand and is obviously related to the 'cross-section' of one strand and the solid angle subtended by the other strand. The 'cross-section' of one strand will remain constant through the cell cycle, but if the distance between the two DNA strands increases, the solid angle subtended by the one strand on the other will decrease, k_o will decrease and so will Δ.

Although the process of DNA replication is not completely understood at present, at the cellular level there exists a hypothesis which is well substantiated by experimental evidence (Okazaki et al 1968, Dupraw 1970, Streffer 1969). The hypothesis proposes that replication takes place in the S phase at many different places along the DNA molecule simultaneously, and that single strand scissions (nicks) are induced by an enzyme so that the double helix can open up, at least partially, to allow the replication process to take place. This process of opening up, or partial unwinding of the DNA double

helix would explain the decrease in the solid angle subtended by the two strands, the decrease in k_o, and thus in Δ, and consequently the decrease in 'a' in the middle of the DNA synthesis phase. This relationship between the variation in the coefficient 'a' and the change in form of the DNA double helix through the S phase is considered to be very strong evidence in favour of the assumption that the double molecular target in the theory is in fact the DNA double helix and that the DNA double helix is the critical target for radiation induced cell killing. This interpretation is further strengthened by the fact that irradiation with argon ions having a very high LET reveals that the 'a' coefficient remains more or less constant (within the errors) throughout the cell cycle (Bird and Burki 1971), Fig. 2L). This is to be expected on the basis of a small variation in distance between the two targets, as the sparsely ionizing radiation will be far more responsive to such a change than the densely ionizing argon ions. The extension of this arguement predicts that the double helix does not open completely in replication as in this case even the argon ions would be expected to reveal a sharp dip in the 'a' coefficient.

The coefficient 'b' demonstrates in general a peak at the G_1-S border, it decreases through S to be low in G_2 and mitosis.

The coefficient $b = pf_o\varepsilon f_1 f_2 n_1 n_2 k_1 k_2 (1-\Delta)^2$ where

- p and f_o are as previously defined
- f_1 and f_2 are the chances that the damaged single targets are repaired
- n_1 and n_2 the number of the single molecular targets
- k_1 and k_2 the respective 'cross-sections' of the single targets

$1 - \Delta$ the fraction of dose going into this mode of damage

ε is the chance that two damaged single targets combine to form a lesion.

Similar arguements apply to p, n_1, n_2 and f_o as have been mentioned previously in the consideration of the 'a' coefficient.

- the parameters k_1 and k_2 represent a physical unit and will remain constant,

- the parameter ε is a geometric factor and will remain constant,

- the parameter $(1 - \Delta)^2$ changes through the cell cycle as Δ changes, but cannot account for the change in 'b' through the cell cycle, probably because with sparsely ionising radiation $(1 - \Delta)$ will be almost 1,

- the parameters f_1 and f_2 are concerned with the repair of single damaged molecular bonds. If the damaged bond is a single DNA break then the repair will be dependent on the enzyme activity, and this may vary through the cell cycle.

In the repair of damaged DNA single strands at least two enzymes are implicated, DNA polymerase, which copies the template DNA strand and replaces the nucleotide (Kornberg 1969, Dupraw 1970) and polynucleotide ligase, which rejoins the phosphate ester bonds (Olivera and Lehman 1967, Olivera et al 1968, Dupraw 1970). The metabolically controlled repair of a damaged single molecular bond, which has been used to quantitatively explain the effect of protracted exposure on cell survival (Leenhouts and Chadwick 1972), has been previously tentatively connected to the metabolically controlled repair of single strand breaks in DNA after radiation and Cleaver (1968) has shown that certain diseased cells which are deficient in one of the

repair enzymes do not give a fractionation effect on radiation and can therefore not repair sub-lethal damage between the fractions. Thus, it would seem that the coefficient 'b' may be linked with the availability and activity of one, or more probably, both the enzymes DNA polymerase and polynucleotide ligase.

The variation of DNA polymerase activity through the normal cell cycle has been described by Streffer (1969). The DNA polymerase is most active at the end of G_1 and beginning of S, decreases through S and is at a minimum in G_2. This is similar to the variation in 'b' through the cell cycle shown in Figure 2 and a reasonable explanation for the variation of 'b' would thus be based upon the availability of the DNA polymerase and probably the polynucleotide ligase for the repair of radiation induced single strand breaks in the DNA. In other words, a competition must take place for the enzymes between the normal cell function and the radiation induced damage.

4. Conclusions

It is concluded that the molecular target theory can be used to analyse the cell survival curve as it varies in shape through the cell cycle, and that the two coefficients in the theory show a periodic variation through the cell cycle. The variation of sensitivity through the cell cycle, which is different in different cell types, may be explained by the combination and relative importance of the two coefficients in the theory.

The variation in the coefficient 'a' is explained on the basis of DNA replication, the variation in the coefficient 'b' can be explained on the basis of the efficiency of the enzymes DNA polymerase

and polynucleotide ligase in the repair of single strand radiation damage.

It is also concluded that the double-target used in the molecular target theory is in fact the two strands of the DNA double helix and that the critical lesion for radiation induced cell death is a double strand break in this molecule.

Acknowledgements

The authors wish to acknowledge the critical discussions which they have had with Prof. R. Braams (Radiation Biophysics Group, Utrecht University) and our colleagues in the institute, especially Dr. K.J. Puite and Drs. W.F. Oosterheert.

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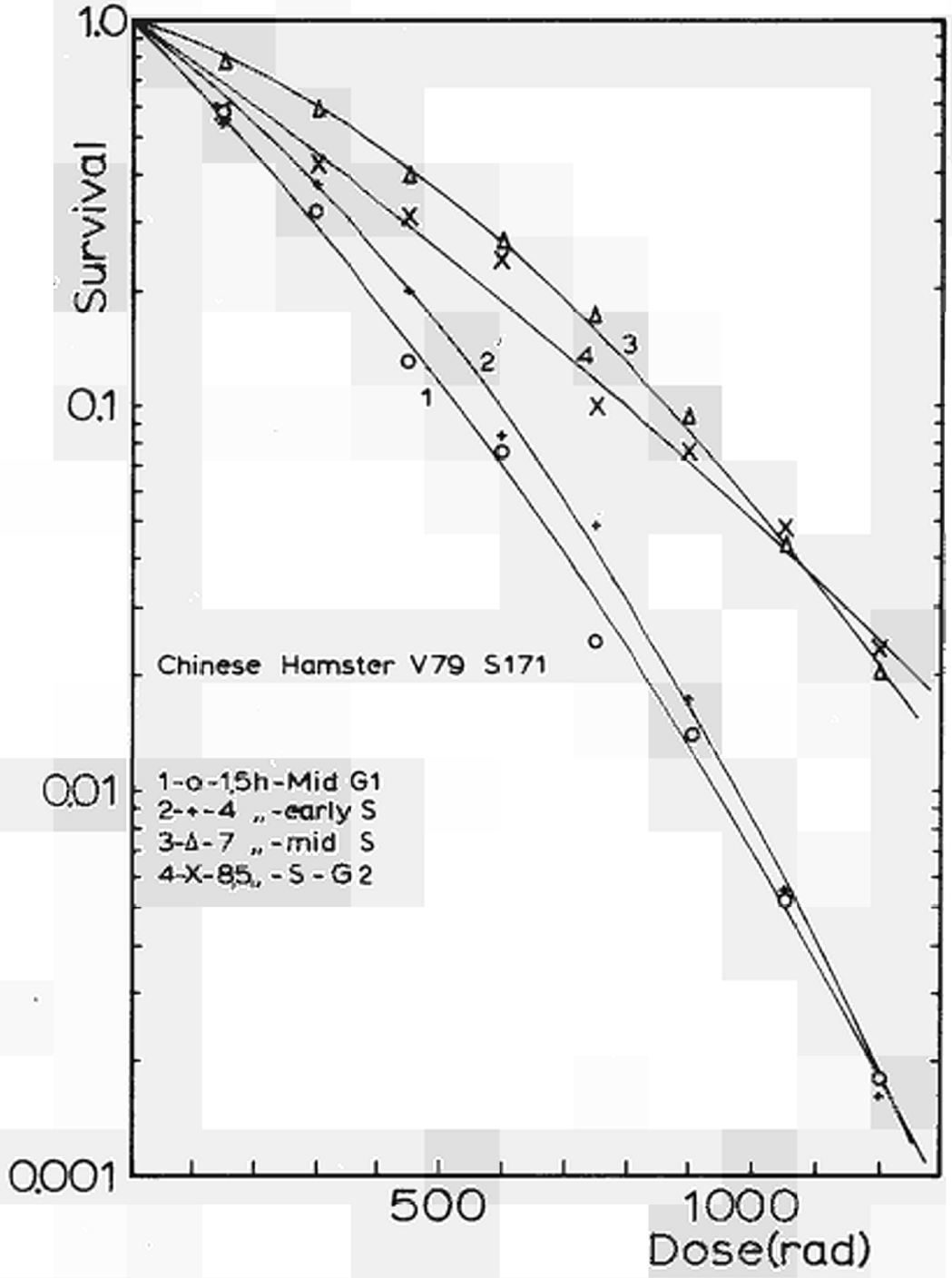

Figure 1 The fitting of an equation $S = e^{-aD}e^{-bD^2}$ to the survival curve of cells synchronised in different parts of the cell cycle.

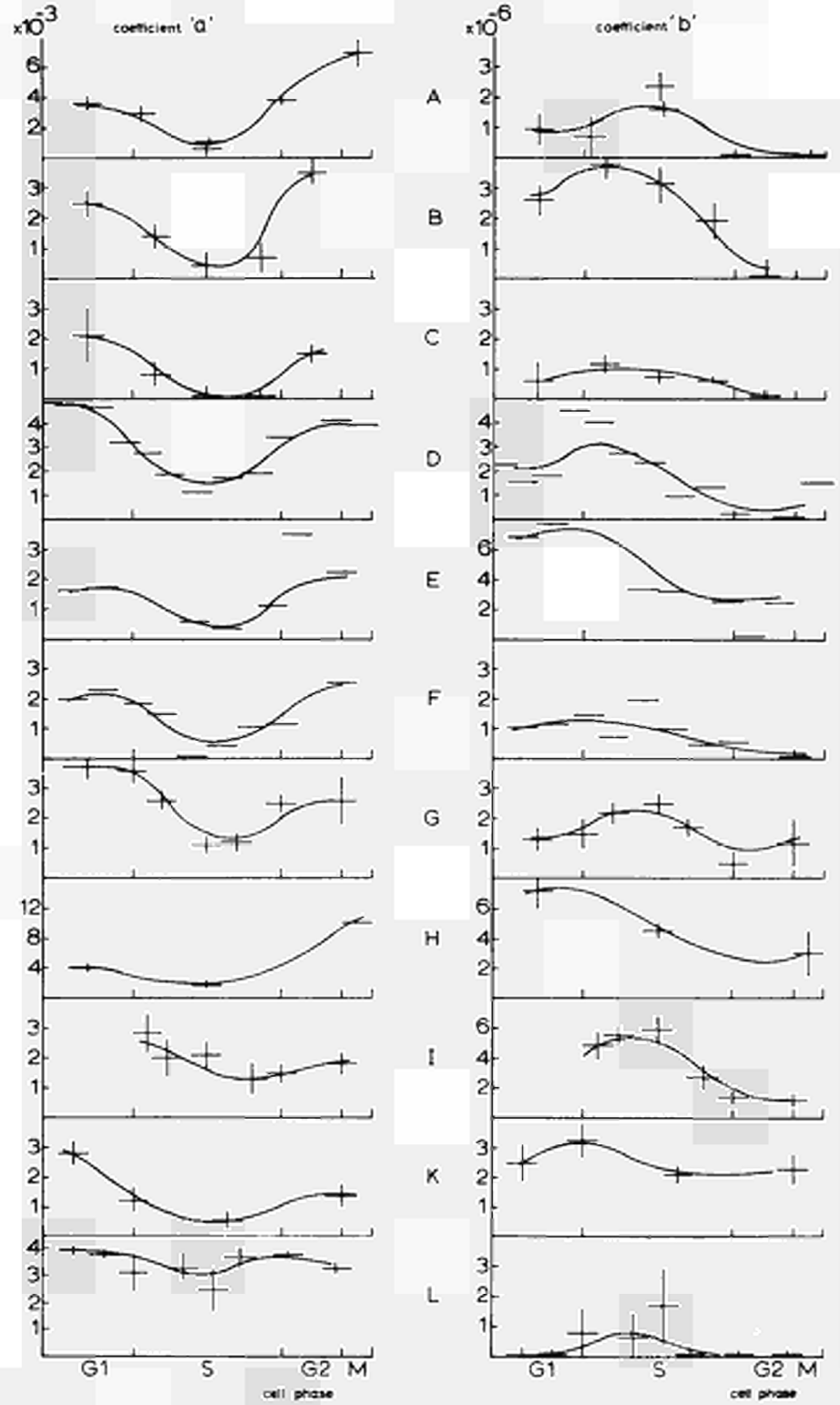

Figure 2 The variation of the coefficient 'a' and 'b' through the cell cycle for different cell lines. Details of the curves are given in Table II.

	Cell line	Irradiation	Reference
A	Chinese hamster V79	250 kVp X-rays 110 R/min.	W.K. Sinclair + R.A. Morton (1966)
B	Chinese hamster V79 - 285B	250 kVp X-rays 160 R/min. in air	J. Kruuv + W.K. Sinclair (1968)
C	id.	id. in N_2	id.
D	Chinese hamster V79 - 325 long G_1	250 kVp X-rays 105 R/min.	W.K. Sinclair (1969)
E	Chinese hamster V79 - 325 long G_1	250 kVp X-rays 160 R/min. in air	J. Kruuv + W.K. Sinclair (1968)
F	id. long G_1	id. in N_2	id.
G	Chinese hamster V79 - S171	250 kVp X-rays 54 R/min.	W.K. Sinclair (1969)
H	Chinese hamster ovary cells	49 keV X-rays 300 rad/min.	W.C. Dewey et al (1971)
I	Human kidney T-cell	250 kVp X-rays 200 R/min.	O. Vos et al (1966)
K	Chinese hamster V79	145 kVp X-rays 190 rad/min.	R. Bird + J. Burki (1971)
L	Chinese hamster V79	Argon ions 2000 keV/μ	id.

Table II. Details of the data which have been analysed and shown in Figure 2.

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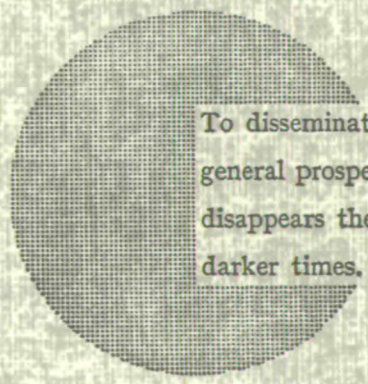

To disseminate knowledge is to disseminate prosperity — I mean general prosperity and not individual riches — and with prosperity disappears the greater part of the evil which is our heritage from darker times.

Alfred Nobel

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