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RADIOBIOLOGICAL CELL SURVIVAL DATA
AND THE PRINCIPLES OF RADIATION DOSIMETRY

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1. INTRODUCTION

Radiation dosimetry for radiation protection purposes may be defined as the measurement of radiation in such a way that the result is proportional to the biological hazard. Existing dosimetric techniques are based on loosely defined concepts which have become accepted due to a lack of any suitable alternative. The quantities that need to be measured to assess radiation hazard should be determined from radiobiological data. For dosimetry purposes, a model describing radiobiological response that does not contradict the data is required, on which to base measurement principles.

The result of many radiobiological experiments depends on observations of effects that depend on biological processes following the initial damage. The simpler the biological system studied, the fewer the number of complicating biological factors involved.

One of the simplest types of radiobiological effect studies where a large amount of experimental data exists*) are those involving the study of the survival probability of single cells. These studies usually take the form of observing the ability of the cells to

*) For a review of published cell survival data, see the paper by Whitmore and Till¹).

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proliferate after irradiation, this being the test of survival. The resulting shape of the survival curve may then be used to deduce information concerning the structure of the radiosensitive sites in the cell using target or hit theories²⁻⁴).

The basic radiobiological processes are complicated, involving unknown parameters. However, if a simple phenomenological description of the average radiation effect could be made based on the simplest assumptions, this could go some way towards determining the quantities to be measured in order to estimate radiation hazard. Furthermore, such a description could be tested with different cell systems, in the hope of determining whether there are any 'biological constants' involved in radiation interaction processes at the cellular level.

2. A MODEL FOR CELL SURVIVAL

The basis for the model of radiation effects in a cell are the following assumptions:

- i) A biological cell contains a number of identical radiosensitive centres or targets.
- ii) The destruction of any one centre results in the death of the cell.
- iii) The centres have one of three possible states:
 - a) normal
 - b) damaged
 - c) destroyed.

The damaged centre in the cell behaves normally and is eventually repaired. Subsequent damage before repair will result in the centre being destroyed.

- iv) The energy that must be deposited in a centre to cause damage must exceed a threshold ΔE_D .
- v) The energy required to kill a centre in a single action must exceed a threshold ΔE_k .

The average number of cell-killing interactions per cell per rad will be

$$\psi_k = \Phi_\ell N P_1 P_k, \quad (1)$$

where

Φ_ℓ is the average number of times a charged particle intersects a cell per rad (1 rad = 100 ergs absorbed per gramme);

N is the number of centres per cell;

P_1 is the probability that if a charged particle intersects a cell it will also intersect a centre;

P_k is the probability that an intersected centre will be destroyed.

For radiation with a linear energy transfer (LET) of ℓ keV/ μ and cells of mass m g and diameter d cm

$$\Phi_\ell = \frac{6.24 \times 10^6 m}{\ell d} \quad (2)$$

$$P_1 = \frac{\text{Effective cross-section of centre}}{\text{Effective cross-section of cell}} = \frac{\sigma_t}{\sigma_o} \quad (3)$$

$$\psi_k = \frac{6.24 \times 10^6 m N \sigma_t P_k}{\ell d \sigma_o} \quad (4)$$

$$= \frac{6.24 \times 10^6 N \sigma_t P_k}{\ell} \quad (5)$$

Similarly, the average number of damaging interactions per cell per rad will be

$$\psi_D = \frac{6.24 \times 10^6 N \sigma_t P_D}{\ell}, \quad (6)$$

where P_D is the probability of damaging an intersected centre.

Suppose that in a cell that has survived a dose D rads, there are n out of the total N centres that are damaged. A further increment of dose δD that does not kill a cell will increase n by δn

$$\delta n = \frac{N - n}{N} \psi_D \delta D . \quad (7)$$

Hence, the average number of damaged centres in a surviving cell at a dose D will be

$$n = \frac{\psi_D}{N} \int_0^D (N - n) dD \quad (8)$$

$$n = N \left[1 - \exp \left(- \frac{\psi_D}{N} D \right) \right] . \quad (9)$$

The probability that a cell which has survived to a dose D will not survive a further dose δD will therefore be:

$$\delta P_s = \left(\psi_k + \frac{n}{N} \psi_D \right) \delta D . \quad (10)$$

The decrease in surviving fraction per dose δD will therefore be given by

$$\delta S = -S \delta P_s = -S \left\{ \psi_k + \psi_D \left[1 - \exp \left(- \frac{\psi_D}{N} D \right) \right] \right\} \delta D \quad (11)$$

from which

$$- \ln S = \left\{ (\psi_k + \psi_D) D + N \left[\exp \left(- \frac{\psi_D}{N} D \right) - 1 \right] \right\} \quad (12)$$

which reduces to

$$- \ln S = N (e^{-aD} + bD - 1) \quad (13)$$

where

$$a = \frac{\psi_D}{N} = \frac{6.24 \times 10^6}{\ell} \sigma_t P_D \quad (14)$$

and

$$b = \frac{\psi_D + \psi_k}{N} = \frac{6.24 \times 10^6}{\ell} \sigma_t (P_D + P_k) . \quad (15)$$

The form of Eq. (13), the survival equation, has the same form as that derived by Haynes⁵⁾ to account for the shoulder in survival curves with a postulation of a re-activating mechanism. The equation applies only to acute irradiations where damage recovery is small during the irradiation time. Equation (7) could be modified to take account of recovery; if the rate of recovery is assumed proportional to the number of centres damaged, Eq. (7) would become

$$\frac{\delta n}{\delta D} = \left(\frac{N - n}{N} \right) \psi_D - \frac{dn}{dt} \frac{dt}{dD} , \quad (16)$$

whence

$$\delta n = \left[\left(\frac{N - n}{N} \right) \psi_D - \frac{\lambda n}{R} \right] \delta D , \quad (17)$$

where λ is a recovery constant and R the dose-rate. However, to include recovery makes the survival equation complicated and introduces another unknown. For the purposes of analysis, the survival equation has two forms:

i) for acute irradiations, no recovery:

$$- \ln S = N (e^{-aD} + bD - 1) ; \quad (18)$$

ii) for chronic irradiations, recovery of damage will be practically complete, making a become small when

$$- \ln S \rightarrow N (b - a) D . \quad (19)$$

3. ANALYSIS OF SURVIVAL DATA

The survival equation can be fitted to published cell survival data. However, an accurate determination of the three free parameters a , b , and N requires a high precision in the results of the biological experiments, as a range of values of the parameters will adequately fit most data.

Barendsen⁶⁾ has given a survival curve of human kidney cells to 250 kV X-rays with some 16 experimental points fitting on to a smooth curve (Fig. 1). Assuming that the dose-rates used were high enough (~ 200 rad/min), this one curve is sufficient to determine N , and hence obtain unique values for a and b for the various radiations used on the same biological system.

The best value for N is determined by effectively eliminating a and b in three simultaneous equations, and is given by (see Appendix):

$$N = \left[\frac{2 \ln S_D - \ln S_{2D}}{2 \sqrt{2 \ln S_D - \ln S_{2D}} - \sqrt{2 \ln S_{2D} - \ln S_{4D}}} \right]^2 \quad (20)$$

where

S_D is the surviving fraction at a dose D ;
 S_{2D} is the surviving fraction at a dose $2D$;
 S_{4D} is the surviving fraction at a dose $4D$.

Using the 250 kV X-ray data, it is found:

$$\begin{array}{ll} D = 100 & N = 5.8 \\ D = 200 & N = 11.0 \\ D = 300 & N = 8.1 \end{array}$$

Hence, a reasonable value for N is taken as 8, independent of D .
 a and b are readily determined (see Appendix):

$$a = \frac{1}{D} \ln \left\{ 1 - \sqrt{\frac{2 \ln S_D - \ln S_{2D}}{N}} \right\}. \quad (21)$$

With $N = 8$ a computed best fit to the 250 kV X-ray data was

$$a = 1.12 \times 10^{-3}$$

$$b = 1.34 \times 10^{-3}.$$

The fit of the survival equation to the experimental points is shown in Fig. 2.

If it is assumed that N is a function of the cell species and of the effect observed only, and a and b depend only on the type of radiation, then at low dose and dose-rates the relative biological effectiveness of the different radiations becomes proportional to $(b - a)$. The values of b , a , and the effectiveness relative to 250 kV X-rays of the radiations used by Barendsen^{6,7}) and plotted in Fig. 1, have been calculated and are listed in the following table.

$$- \ln S = N(e^{-aD} + bD - 1)$$

$$N = 8$$

Radiation	Quoted LET keV/ μ	a $\times 10^{-3}$	b $\times 10^{-3}$	b/a	$b - a$ $\times 10^{-3}$	RBE*)
250 kV X-rays	-	1.10	1.31	1.19	0.21	1.0
14.9 MeV deuterons	5.6	0.95	1.28	1.34	0.33	1.58
3.0 MeV "	20	0.59	1.29	2.19	0.70	3.35
26 MeV α s	25	0.87	1.54	1.77	0.67	3.20
8.3 MeV α s	61	-	-	-	1.46	7.0
5.1 MeV α s	88	-	-	-	1.98	9.4
4.0 MeV α s	110	-	-	-	2.2	10.5
3.1 MeV α s	141	-	-	-	2.00	9.5
2.5 MeV α s	165	-	-	-	1.54	7.3
1.8 MeV α s	200	-	-	-	1.28	6.1
Sr + Y ⁹⁰ β s	-	0.73	1.00	1.36	0.265	1.26
200 kV X-rays	-	1.01	1.30	1.30	0.29	1.38

*) RBE = biological effectiveness relative to 250 kV X-rays.

4. SIZE OF THE TARGET

A rough order of magnitude determination of the size of the biological target may be inferred from the values of the parameters. For low specific ionization radiations, the ion column approaches a line along which are individual ionizations. In this case, the intersection cross-section of the target approaches its geometric area. As the interaction model suggests only one degree of damage, and this model fits the experimental data, it could be argued that the damage is in fact one ionization, and lethality a double ionization. If this is the case, then at low LET, P_D and P_K will be the probabilities of obtaining one and two ionizations over a length of track δx Å equal to the average chord length through the target. For radiation of LET ℓ keV/ μ , the average number of ionizations, each requiring an energy deposition of ω eV over a length δx Å, will be m , where

$$m = \frac{0.1 \ell \delta x}{\omega} \quad (22)$$

Provided that the ionization events are independent of each other, the probabilities will be given by the Poisson coefficients, namely:

$$P_D = m e^{-m} \quad (23)$$

$$P_D + P_K = 1 - e^{-m} \quad (24)$$

$$b/a = \frac{P_D + P_K}{P_D} = \frac{1 - e^{-m}}{m e^{-m}} = \frac{e^m - 1}{m} \quad (25)$$

For 250 kV X-rays $b/a = 1.19$, from which $m = 0.34$ and, using Eqs. (23) and (24), $P_D = 0.24$, $P_K = 0.046$. Assuming $\ell = 3.5$ keV/ μ and $\omega = 30$ eV, then $\delta x \sim 30$ Å.

The values of ℓ and ω are mere guesses but should give an order of magnitude estimation of δx . The cross-section of the target σ_t is obtained from Eq. (14)

$$\sigma_t = \frac{a \times l}{6.24 \times 10^6 P_D} \quad (26)$$

$$= 2.6 \times 10^{-9} \text{ cm}^2 . \quad (27)$$

As one dimension of the target is of the order of $30 \text{ \AA}$, the picture given by the data is of a target in the form of a filament, tens of $\text{\AA}$ in diameter and of the order of 100 microns in length; or the target is in the form of a membrane of about 0.2 square microns and $30 \text{ \AA}$ thick. The mass of the target corresponds to a molecular weight of about 5×10^8 . There are eight such targets per cell, and two ionizations anywhere inside one of these targets will result in a loss of the proliferation capacity of the cell.

5. DISCUSSION

The derived expression for cell survival shows a good fit to the form of the survival curves used, from which values of the three free parameters a , b , and N can be determined. In order to use the theory to construct a model of radiation interaction, it is necessary to show that N is independent of the radiation, and depends only on the biological system used and the tests performed. If it could be assumed that radiation interaction with biological materials was always associated with some critical distance (the average path length through the target), then the ratio b/a should be independent of the biological system and depend only on the radiation. The precision of available survival data does not allow either of these assumptions to be tested. Hence the theory can only be used as a tentative first attempt at formulating the parameters involved in radiobiological damage processes.

The amount of radiation damage at low dose and dose-rates (the fraction of cells killed) will be given by

$$1 - S = N(b - a)D . \quad (28)$$

Using Eqs. (14) and (15), this becomes

$$(1 - S) = \psi_k D, \quad (29)$$

where ψ_k is the average number of "killing" interactions per cell per rad. Hence a model to use as a basis for radiation measurement principles would be to measure a quantity proportional to the number of "killing" interactions per cell. This would be achieved by measuring the frequency per unit mass with which the energy density along the track of charged particles exceeds that necessary for killing a target. On the basis of the deductions made, if a measurement was carried out of the number of times two or more ionizations were deposited in tissue within $30 \text{ \AA}$ of each other, this would be directly proportional to the amount of biological damage independent of the type of radiation.

The important quantity governing the response of the detector is the value of $\delta x/\omega$, in this case $\approx 10^{-8} \text{ g/cm}^2/\text{eV}$, where ω is the energy required to produce a unit of an observable effect in a detector. According to theory, it is this quantity that determines the shape of the LET-RBE curve. For radiation protection purposes, the relative effectiveness of radiation as a function of LET has been laid down by the International Commission of Radiological Protection⁸⁾. Similar assumptions as used in this theory when applied to the ICRP curve defined as the maximum effective radiation is 20 times as effective as radiation of LET. $3.5 \text{ keV}/\mu$ leads to a value of $\delta x/\omega \approx 10^{-9} \text{ g/cm}^2/\text{eV}$ ⁹⁾.

The conclusions drawn from the theory about the interaction model are, of course, a direct consequence of the initial assumptions; although it is shown that the model is consistent with a set of biological data, it would be necessary to devise radiobiological tests of the theory before it can have any real value.

6. CONCLUSIONS

One set of survival data can be fitted to an equation

$$- \ln S = N(e^{-aD} + bD - 1) .$$

The interpretation and numerical values of the parameters N , a , and b are determined from the theory. It is suggested that N is a function of the cell and the biological tests made and that the ratio b/a is a function of the radiation causing the biological damage. The above equation is derived assuming acute irradiation conditions and may be reduced to apply to chronic conditions to become:

$$- \ln S = N(a - b)D .$$

The theory suggests that if a measurement is made of the frequency with which an ionization density of two or more ions per distance δx occurs, this would be proportional to the damage independent of the type of radiation. δx is related to ω , the energy loss per ion pair in the detector, by $\delta x/\omega = 10^{-8}$ to 10^{-9} g/cm²/eV.

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APPENDIX

REDUCTION OF THE SURVIVAL EQUATION

N can be expressed in terms of survival only by effectively eliminating a and b in three simultaneous equations using different values of dose. This was done in the following way:

$$- \ln S_D = N \left[e^{-aD} + bD - 1 \right] \quad (A1)$$

$$- \ln S_{2D} = N \left[\left(e^{-aD} \right)^2 + 2 bD - 1 \right] \quad (A2)$$

$$\therefore \frac{2 \ln S_D - \ln S_{2D}}{N} = \left[\left(e^{-aD} \right)^2 - 2 e^{-aD} + 1 \right] \quad (A3)$$

$$= \left(e^{-aD} - 1 \right)^2 \quad (A4)$$

$$\therefore e^{-aD} - 1 = \pm \sqrt{\frac{2 \ln S_D - \ln S_{2D}}{N}} \quad (A5)$$

As a is real and positive, the sign of the square root must be negative. Put Eq. (A5) in Eq. (A1)

$$\frac{- \ln S_D}{N} = b D - \sqrt{\frac{2 \ln S_D - \ln S_{2D}}{N}} \quad (A6)$$

and

$$\frac{- \ln S_{2D}}{N} = 2 b D - \sqrt{\frac{2 \ln S_{2D} - \ln S_{4D}}{N}} \quad (A7)$$

$$\therefore \frac{1}{N} \left(2 \ln S_D - \ln S_{2D} \right) = \frac{1}{\sqrt{N}} \left[2 \sqrt{2 \ln S_D - \ln S_{2D}} - \sqrt{2 \ln S_{2D} - \ln S_{4D}} \right] \quad (A8)$$

$$\therefore N = \left[\frac{2 \ln S_D - \ln S_{2D}}{2\sqrt{2 \ln S_D - \ln S_{2D}} - \sqrt{2 \ln S_{2D} - \ln S_{4D}}} \right]^2 \quad (A9)$$

Value of a

From Eq. (A5)

$$a = \frac{1}{D} \ln \left[1 - \sqrt{\frac{2 \ln S_D - \ln S_{2D}}{N}} \right] \quad (A10)$$

Value of b

From Eq. (A1)

$$b = \frac{1}{D} \left\{ \frac{-\ln S_D}{N} + 1 - e^{-aD} \right\} \quad (A11)$$

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Cell survival curves from Barendsen's paper (Ref. 6)

Fig. 1

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Points experimental Barendsen 250 keV X-rays

Line obtained from $-\ln S = N(e^{-aD} + bD - 1)$
with $N = 8$

$$a = 1.116 \times 10^{-3}$$

$$b = 1.34 \times 10^{-3}$$

Fig. 2

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