A COMMON MOLECULAR MECHANISM IN RADIOBIOLOGY ITS IMPLICATIONS IN RADIOLOGICAL PROTECTION

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Abstract

The paper sketches the development of a theoretical model which attempts to describe the biological action of ionizing radiation starting from a molecular mechanism through the cellular effects to the effects occurring in animals. The model is based on the action of radiation on the DNA molecule in the cell and it is assumed that radiation induced DNA double strand breaks are the most critical lesions in cellular radiobiology.

In the first part of the paper the molecular mechanism of DNA double strand breakage is described and related to cellular effects such as radiation induced cell death and radiation induced mutations. Evidence is presented to show that cell death, mutations and DNA double strand breaks all have the same dose kinetics.

The effects of dose rate and LET on DNA double strand breakage and on cell death and mutation induction are considered and the original equations can be modified to give the form expected at low dose and low dose rate. Using the basic equations a limiting relative biological effectiveness is defined which is dose and dose rate independent and is particularly relevant in radiological protection.

In the second part of the paper the cellular effects are tentatively related to effects occurring in animals and organs such as animal survival and radiation induced cancer. The theoretical equations are compared with experimental data in the literature.

The consequence of the mechanism for the genetic effects of radiation are briefly discussed with regard to the radiation dose kinetics.

It is concluded that the model gives dose relationships which are non-linear but contains a means of extrapolating from high dose, high dose rate results to obtain parameters which could be of importance for radiological protection. It suggests that a basic mechanism may be common to the various radiobiological effects and to all eukaryotic cells and it would thus provide a basis for the extrapolation of results in animals to man. The model also provides a common link between the biochemistry of DNA, the metabolism of the cell, radiobiology and radiological protection.

Introduction

Recently a molecular theory of cell survival has been proposed[1] which attempts to explain the dose relationship of cell survival following ionizing radiation on the assumption that radiation induced DNA double strand breaks are the most critical radiation damage. The application of this molecular model to the dose rate effect and the analysis of the survival of cells synchronized at different phases of the cell cycle has given results which can be interpreted on the basis of the biochemistry and behaviour of the DNA molecules in the cell.

It is somewhat surprising that if the molecular model is extended to describe radiation effects on more complex systems certain interesting correlations can be found which indicate that a common molecular mechanism may be at the root of these radiation effects.

The model is still in its infancy, further developments and refinements are necessary and it is clear that more experimental work must be carried out to correlate the molecular mechanism more closely with the observed effect. However, an attempt will be made here to sketch the relationship of the various radiation induced effects via the induction of the common primary damage, to show that these dose relationships are compatible with experimental results and to indicate that the interpretation of the relationships has a biological significance. Finally, the implications of the molecular model for radiological protection are inferred.

The proposed Molecular Mechanism

It is generally, though not universally, accepted that the DNA molecule is the most critical target for radiation. The DNA occurs in the nucleus of the cell as chromosomal DNA in the form of a long double helix structure and the induction of single strand breaks and double strand breaks in the DNA helix by radiation is a well documented scientific fact[2,3].

The basic assumption of the theoretical model presented in this paper is that the double strand break is the critical lesion which eventually leads to the biological end effects.

Radiation can induce a double strand break in the DNA double helix in two ways

1. a double strand break in one radiation event,
2. a double strand break as a result of the combination of two independent breaks from two independent radiation events.

Following a radiation dose D the number of double strand breaks produced in one radiation event in a cell is proportional to dose, say αD. The number of double strand breaks produced in two independent radiation events in a cell is proportional to the square of the dose, say βD^2. Thus the total number of DNA double strand breaks in a cell is given by

$$N = \alpha D + \beta D^2. \qquad (1)$$

In the original derivation of this expression[1] the coefficients α and β contain parameters which have a radiobiological significance and it is important that in the analysis of the experiments this radiobiological significance be borne in mind.

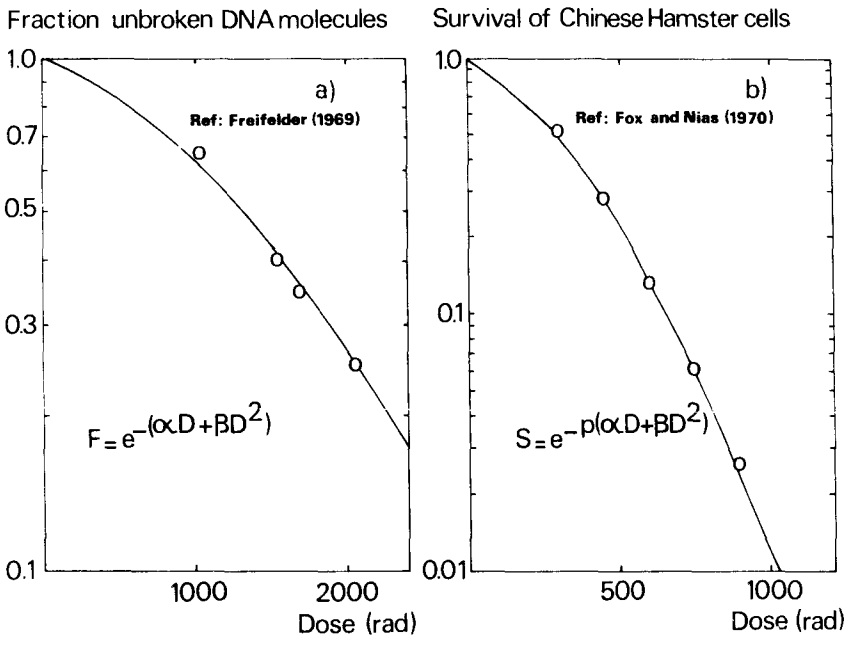
Cellular Effects

Cell Survival

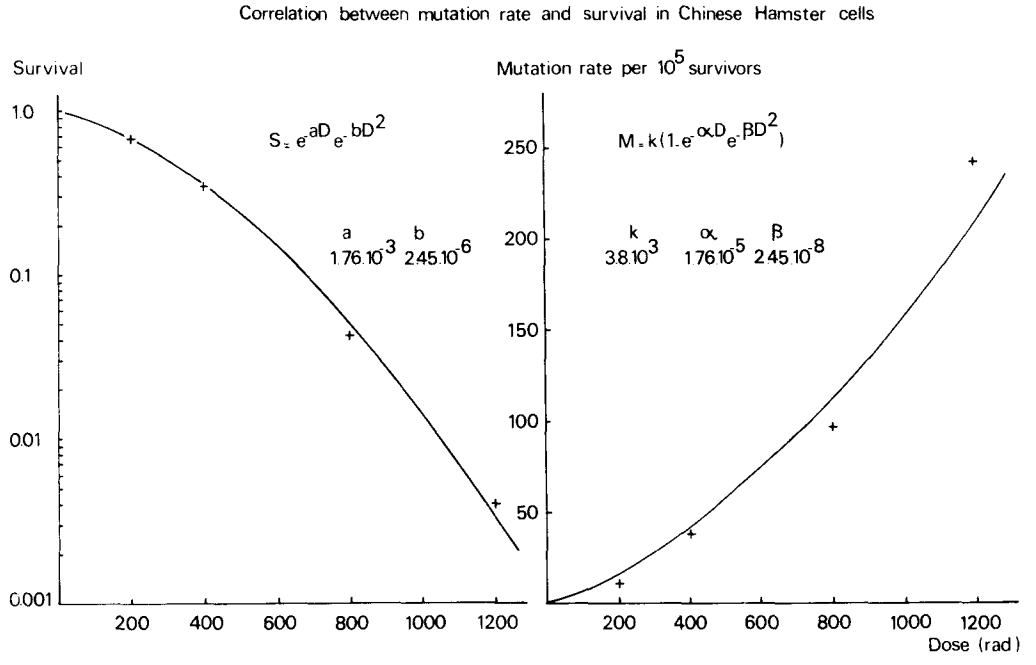
In a population of cells the number of DNA double strand breaks N will be the mean value of a poissonian distribution. If each double strand break has a probability p of leading to cell reproductive death, then poissonian statistics can be used to derive the probability that N double strand breaks lead to cell reproductive death and cell survival is given by

$$S = e^{-pN} = e^{-p(\alpha D + \beta D^2)} \qquad (2)$$

In figure 1 the induction of DNA double strand breaks and cell survival are shown analysed according to the model presented here. The figure illustrates that the dose kinetics are the same. If the coefficients are calculated to give the chance for a double strand break per nucleotide pair then the magnitude of the coefficients is also very similar ($\alpha^1 = 1.6\ 10^{-12}$, $p\alpha^1 = 5.3\ 10^{-13}$ rad^{-1}; $\beta^1 = 1.6\ 10^{-15}$, $p\beta^1 = 9.3\ 10^{-16}$ rad^{-2}).



1. Correlation between dose kinetics for radiation induced DNA double strand breaks and cell survival (ref. 2, 21).



2. Correlation between dose kinetics and coefficients for radiation induced mutations and survival in chinese hamster cells. (ref. 4).

Mutation Induction

Radiation damage of the DNA in the cell may lead to a change in the hereditary material which may be expressed as a mutation when the cell divides. A DNA single strand break can be perfectly repaired as the repair enzymes can copy the undamaged complementary strand. The repair of a DNA double strand break however, is, if it is possible, more likely to be accompanied by a fault and is thus a possible source of mutations. An unrepaired double strand break may lead to a loss of DNA and also to a mutation.

If each DNA double strand break in the cell has a probability q of leading to a specific mutation then the probability that N double strand breaks in the cell lead to the incidence of specific mutations is

$$M = 1 - e^{-qN} = 1 - e^{-q(\alpha D + \beta D^2)} \qquad (3)$$

Figure 2 demonstrates the correlation between cell survival and mutation induction in chinese hamster cells[4]. The coefficients derived for cell survival can be used to give the curve for mutation induction. The dose kinetics and the correlation with cell killing suggest that the mutations arise from a DNA double strand break.

Dose rate and LET effects

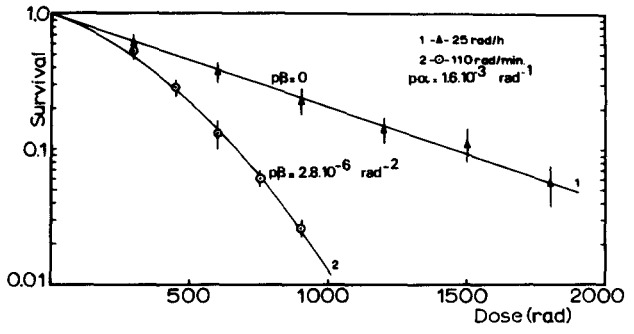
According to the model the dose rate and LET effects occurring in cell survival and mutation induction are directly related to the dose rate and LET effects occurring in the production of DNA double strand breaks. These effects can be explained briefly in the following way. The induction of double strand breaks in one radiation event is independent of any dose rate effect. The occurrence of double strand breaks arising from two single strand breaks will be dependent on the dose rate of the radiation if the DNA single strand breaks can be repaired during irradiation. In general cells have the ability to repair a single strand break accurately by the action of specific enzymes. Consequently, as the radiation dose is protracted more single strand breaks will repair and fewer double strand breaks will arise from two independent radiation events. This means that as the dose rate is decreased the coefficient β will decrease and at low enough dose rates will eventually become zero and equation (2) and (3) can be modified accordingly. This effect is illustrated in Figure 3a for cell survival.

The induction of double strand breaks in one radiation event whilst independent of dose rate is not independent of radiation quality and more densely ionizing radiation will have a greater chance of breaking both strands in one event than sparsely ionizing radiation. This effect is illustrated in Figure 3b for cell survival. The neutron irradiation is more efficient in breaking the double strand in one event and the $p\alpha$ coefficient is consequently larger.

The model implies that the coefficient $p\alpha$ or $q\alpha$ is of prime importance for radiological protection as it is the coefficient which determines the biological effect at low doses and at low dose rates. The model based on the mechanism of DNA double strand breakage presented here offers the possibility of estimating the $p\alpha$ or $q\alpha$ coefficient from experiments made at high doses and high dose rates. It is possible to define a limiting relative biological effectiveness (RBE_o) using the α coefficients derived for two different radiations as

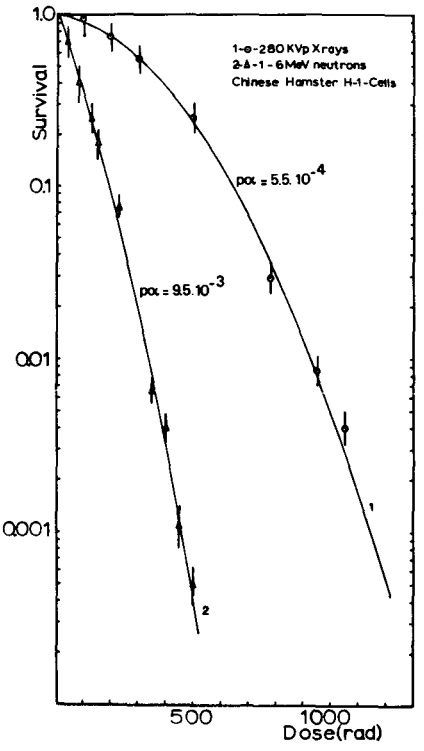
$$RBE_o = \frac{\alpha_{\text{test radiation}}}{\alpha_{\text{standard radiation}}} \qquad (4)$$

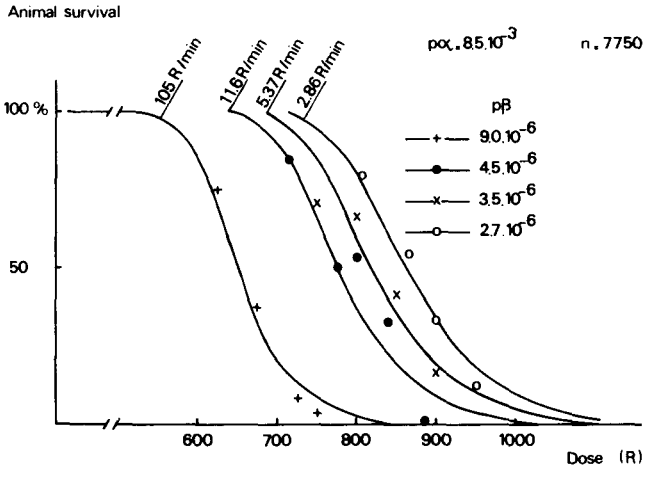
This RBE_o is especially relevant at low doses and dose rates, is dose and dose rate independent and its value is important in determining appropriate Quality Factor values.



3. a. Dose rate effect on cell survival (ref. 21).
   25 rad/h $p\alpha = 1.6\times10^{-3}$ $p\beta = 0$
   110 rad/min $p\alpha = 1.6\times10^{-3}$ $p\beta = 2.8\times10^{-6}$

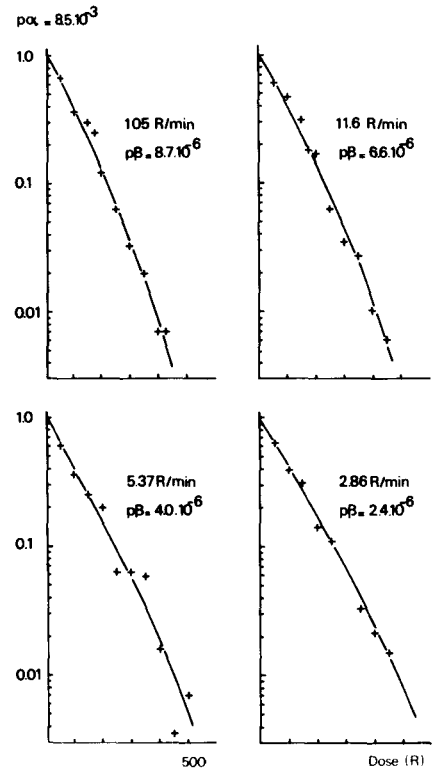
   b. (right) Effect of radiation quality on cell survival (ref. 22).
   280 kVp X $p\alpha = 0.55\times10^{-3}$ $p\beta = 4.8\times10^{-6}$
   1-6 MeV n $p\alpha = 9.5\times10^{-3}$ $p\beta = 12.3\times10^{-6}$
   $RBE_o = 17$





4. Fit of the equation $L = 1 - [1 - e^{-p(\alpha D+\beta D^2)}]^n$ to the survival of female mice irradiated with X-rays at different dose rates (see ref. 7).

5. (right) Fit of equation $S = e^{-p(\alpha D+\beta D^2)}$ to the survival of transplanted mouse bone marrow cells irradiated <u>in vivo</u> in recipient mice at different dose rates.



Multicellular Effects

In this section the associations made between the molecular mechanism and the cellular effects are tentatively extended to develop dose relationships for multicellular effects. In this extension it is necessary to assume that the multicellular effect arises from the accumulation of damage at the cellular level and that in a homogeneous population the chance that the primary radiation damage develops to the ultimate biological effect is the same for all the animals in the experiment.

Animal Survival

If it is assumed that:

1. the death of an animal following radiation is associated with a specific radiation syndrome which is connected with the killing of cells from a critical organ. For instance the LD 50/30 in mice is associated with the bone marrow syndrome which is related to the survival of haemopoietic stem cells [5,6].
2. the death of the animal results because the specific cell pool is reduced below a critical level. In a homogeneous animal population this amounts to killing a certain critical number of the specific cells.

Then, if the specific cell survival is given by

$$S = e^{-p(\alpha D + \beta D^2)}$$

and n is the critical number of cells, the chance that the animal will survive is given by

$$L = 1 - (1 - e^{-p(\alpha D + \beta D^2)})^n \qquad (5)$$

The survival curve for animals is thus defined by three coefficients, $p\alpha$, $p\beta$ and n, two of which define cell survival, and are related to the induction of double strand breaks in the DNA molecule.

A practical application of this theoretical expression to the survival of animals and of the correlation with cell survival is demonstrated in Figures 4, 5 and 6. Figure 4 presents the fit of equation (5) to the survival of female $B_6D_2F_1$ mice aged 10-14 weeks and weighing 18-24 g following X-irradiation at different radiation dose rates[7]. Figure 5 presents the survival of transplanted bone marrow cells, irradiated *in vivo* in recipient mice at the same dose rates, fitted with equation (2). The analysis has been made as follows:

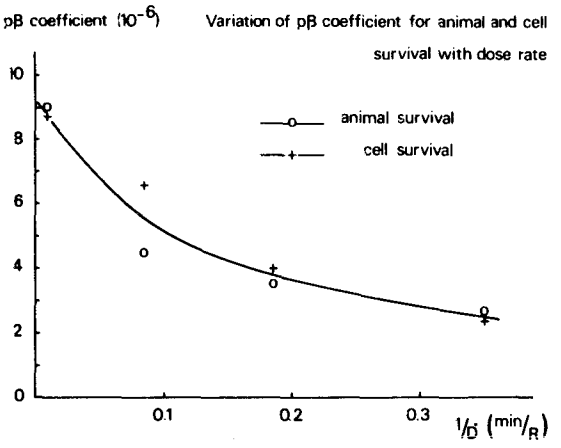
1. A best fit of equation (2) was made to the cell survival curves. The $p\alpha$ coefficient was found to be approximately constant: $(7.9 - 8.9) \times 10^{-3}$.
2. A mean value for $p\alpha$ of 8.5×10^{-3} was used and the best $p\beta$ coefficients were determined for the cell survival.
3. Using $p\alpha = 8.5 \times 10^{-3}$ the animal survival curves were best fitted for different n values to see if the $p\beta$ values were in the same order as those found for cell survival.
4. This was indeed found and n was fixed at n = 7750, $p\alpha = 8.5 \times 10^{-3}$ and the best $p\beta$ values were determined.

Figure 6 gives the comparison between the $p\beta$ values determined in the cell survival studies and in the animal survival studies at the different dose rates.

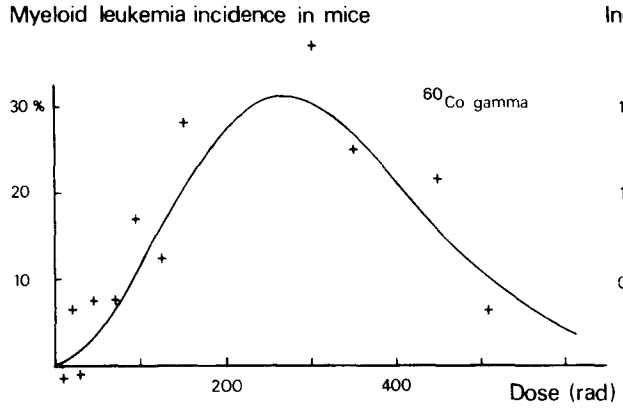
Radiation Induced Cancer

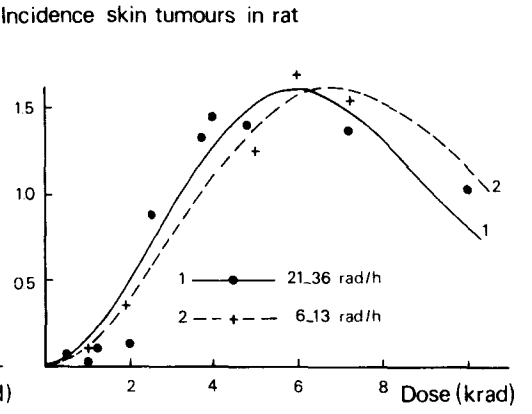
If it is assumed that a somatic mutation is the radiation induced process which eventually leads to the development of a radiation induced cancer[8,9,10] and that the mutated cell must be able to divide to give rise to the cancer[11,12] then the considerations on cell survival and mutation induction can be combined and an equation for cancer induction can be derived from equations (2) and (3) to give:

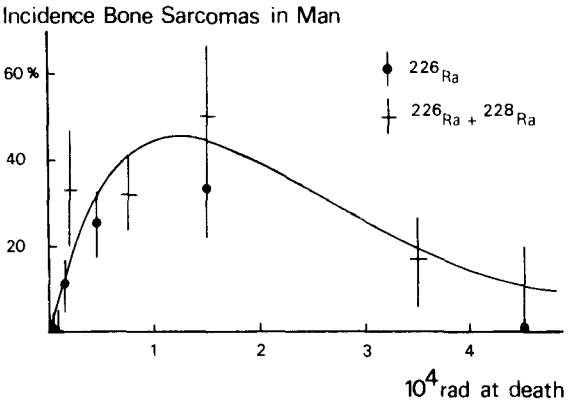
$$C = (1 - e^{-m(\alpha D + \beta D^2)})\, e^{-p(\alpha D + \beta D^2)} \qquad (6)$$



6. Comparison of the variation in the pβ coefficients derived from animal and cell survival studies with irradiation dose rate (see ref. 7).







7. The fitting of the equation $C = k(1 - e^{-m(\alpha D+\beta D^2)})e^{-p(\alpha D+\beta D^2)}$ to the incidence of myeloid leukemia in male mice (a) (ref. 13), skin tumours in rats (b) (ref. 14) and bone sarcomas in man (c) (ref. 15). k is a normalisation constant

	k	mα	mβ	pα	pβ
a	1.25	2.0×10^{-4}	9.0×10^{-6}	2×10^{-4}	9.0×10^{-6}
b 1	0.6	7.2×10^{-6}	1.8×10^{-8}	8×10^{-6}	2.0×10^{-8}
b 2	0.6	7.2×10^{-6}	1.35×10^{-8}	8×10^{-6}	1.5×10^{-8}
c	3.5	3.0×10^{-5}	0	7×10^{-5}	0

where m is the probability that a double strand break leads to a specific cancer mutation. Figure 7 shows the fitting of this equation to various data on cancer incidence. Figure 7a shows the equation fitted to the incidence of myeloid leukemia in male mice following ^{60}Co gamma irradiation[13]. In figure 7b the effect of dose rate is demonstrated for the incidence of skin tumours in rats[14], the two curves being fitted by varying the β coefficient only. In figure 7c an example of the effect of densely ionizing radiation on the incidence of bone sarcomas in man[15] is given using equation (6) with the β coefficient held at zero.

At low doses and low dose rates equation (6) reduces to

$$C = (1 - e^{-m\alpha D})\, e^{-p\alpha D} \qquad (6a)$$

This expression is linear from the origin, saturates to a maximum and then decreases. The results of the Oxford Survey provide a dose relationship for childhood cancer following obstetric radiography[10] at very low doses of radiation which can be compared with equation (6a). In figure 8 two examples of a possible fitting of equation (6a) to the dose response are presented. Curve number 1 is based on the assumption that the cell sensitivity of the foetus is similar to mammalian cells and gives a more or less linear fitting. Curve number 2 is based on the assumption that the foetus is more sensitive than normal cells and a clear curvature[16] is obtained. It is important to realise that the comparison presented in Figure 8 has certain short-comings the most important of which is that all cancers are considered together whereas the equation should be applied to specific cancers. The two curves in Figure 8 should not be considered as the only possibilities or even the extreme possibilities but only as a demonstration of the agreement between experimental results and the theory.

Some important comments can be made on the basis of equations (6) and (6a). Even at low doses there will be no threshold, and for sparsely ionising radiations a reduction in dose rate will lead to a sparing effect. At high LET, of course, equation (6a) applies for all dose rates and no sparing effect will be observed. Furthermore a specific mathematical feature of equation (6) is that the peak height is independent of the coefficients α and β and only depends on the relationship between p and m; this is demonstrated in figure 7b. The consequence of this is that for a specific cell type the maximum incidence is independent of irradiation type and conditions although the dose at which the peak occurs is dependent on these factors.

Genetic Effects

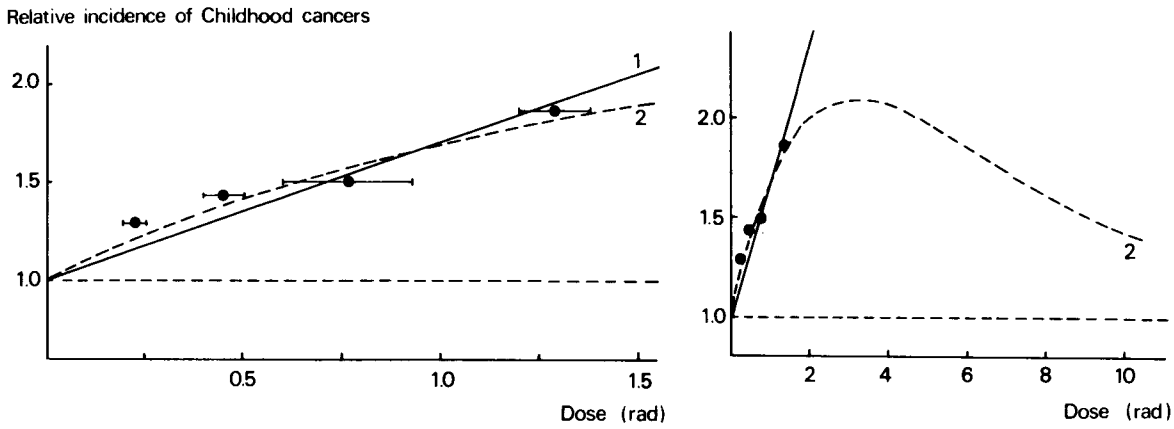
No attempt will be made in this section to consider the multitude of problems involved in the estimation of genetic risk, but an attempt will be made to demonstrate that in two different stages of the same cell, the drosophila oocyte, where the radiation induction of mortality has apparently different dose kinetics[17,18], the same process of DNA double strand breakage could be involved.

Figure 9 shows the mortality in Drosophila oocytes irradiated in stage 7 and stage 14. The curve for stage 14 oocytes has understandably been interpreted as an exponential curve; however, if the curve is analysed using the model and the equation

$$S = e^{-p(\alpha D + \beta D^2)},$$

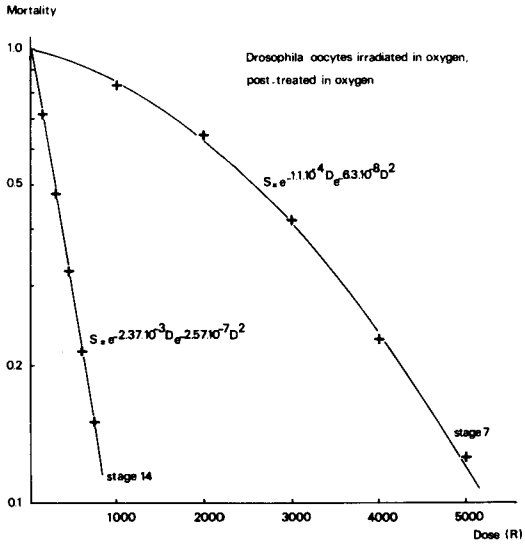
then the best fit does lead to a positive $p\beta$ coefficient. This indicates that in this stage of the cell the induction of 'double strand breaks in one radiation event' is a dominant process but that the same mechanism of DNA double strand breakage could be involved in the induction of mortality in both stages of the Drosophila oocyte.

The induction of DNA double strand breaks is a process which can occur in all eukaryotic cells and it should therefore be possible to describe radiation effects in the reproductive cells of other insects and animals by the same dose



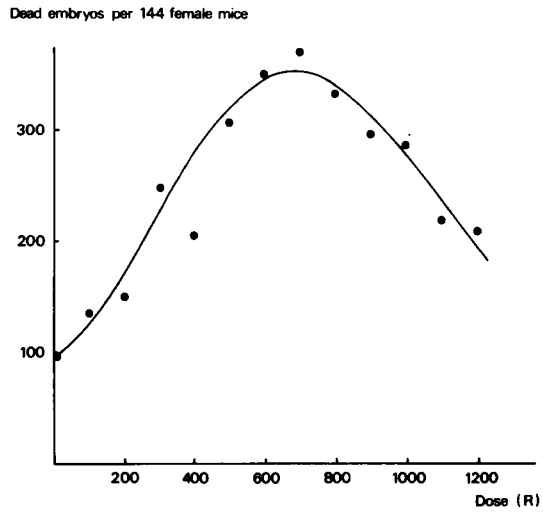
8. The fitting of the equation $C = k(1 - e^{-m\alpha D}) e^{-p\alpha D}$ to the relative incidence of all cancers in children following obstetric radiography (ref. 10). k is a normalisation constant.

	k	mα	pα
Curve No. 1	320	2.2×10^{-3}	2.2×10^{-3}
Curve No. 2	4.38	2.2×10^{-1}	2.2×10^{-1}



9. The mortality of Drosophila oocytes, stage 7 and stage 14, fitted with the equation $S = e^{-p(\alpha D + \beta D^2)}$: (ref. 17,18)

	pα	pβ
Stage 7	1.06×10^{-4}	6.3×10^{-8}
Stage 14	2.37×10^{-3}	2.57×10^{-7}



10. The relationship between a measure of the dominant lethality in mice and radiation dose (ref. 19) fitted by the equation

$DE. = K (B + 1 - e^{-q(\alpha D + \beta D^2)}) e^{-p(\alpha D + \beta D^2)}$

K	B	qα	qβ
1029	97/1029	2×10^{-4}	1.25×10^{-6}

pα	pβ
1.6×10^{-4}	1×10^{-6}

kinetics. An attempt to demonstrate this has been made in figure 10 which presents the relationship between a measure of the dominant lethality in mice and the radiation dose[19]. The experimental points represent the number of dead embryos per 144 female mice found following mating with male mice which had received a local testicular irradiation. The equation which has been fitted is

$$DE = K\ (B + 1 - e^{-q(\alpha D + \beta D^2)})\ e^{-p(\alpha D + \beta D^2)}$$

where K is the number of implantations, and B represents the dead embryos found in the unirradiated control. The spermatozoa responsible for these dead embryos can also be 'inactivated' by the radiation.

This figure shows that the same dose kinetics are also found in the mouse cells as well as in the Drosophila cells. These kinetics do contain a dose rate effect which is in accordance with the findings quoted in Unscear[20] that the induction of genetic effects are lower following chronic irradiation.

Discussion

In this paper the development of a theoretical model to describe the effect of radiation on a series of biological end points has been briefly sketched.

The model is based on one critical radiation induced lesion which is known to occur in cells following radiation, the DNA double strand break. Correlations have been presented between the induction of DNA double strand breaks in vitro and cell survival; between cell survival and mutation induction; between cell survival and animal survival; between mutation induction and cell survival and cancer induction; and it has been shown that the same dose kinetics are involved in the induction of genetic effects.

We would like to emphasize the aspects of the model which we consider to be most important. The fit of the various equations to the experimental results is satisfying but is in itself not as important as the fact that the proposed molecular mechanism, which will be caused in all eukaryotic cells by radiation, forms a common thread which links the different biological effects. The dose kinetics and LET and dose rate effect arising through the mechanism are all reflected in the dose kinetics, LET and dose rate effects of the various biological end points. The second important aspect of the model is that it offers the possibility of interpreting multicellular and cellular biological effects on the basis of a common radiation induced molecular lesion.

The dose kinetics, the explanation of the dose rate effect and the LET effect developed in this paper are strongly reminiscent of the classical theory of chromosome exchange aberrations. It is important, however, to realize that the theory presented here is essentially different from the classical theory both in the starting assumptions and in the interpretation. The theory presented here starts from the assumption that the critical radiation lesion occurs at the molecular level and not the chromosome level, and the interpretation of the variations in the radiobiological effects is based on the biochemistry and metabolism of the DNA in the cell. The evidence in support of the assumption that the DNA double strand break is the critical lesion is the close agreement found between the coefficients determined for cell survival and the induction of DNA double strand breaks in vitro, and the fact that the analysis of the survival of synchronized cells at different stages of the cell cycle gives a variation in the $p\alpha$ coefficient which is in accordance with the partial separation of the DNA strands at the replication forks during the synthesis phase[1].

It is clear that the development of the model from the molecular mechanism to the cellular and animal effects is theoretical and although it is based on assumptions which have been suggested previously, such as the somatic mutation theory of cancer, it is also somewhat speculative. The compatibility of the equations with both the experimental results and the proposed mechanism plus the coherence of the interpretation which can be obtained via the model are promising and it should not be too difficult to design experiments to test the

model more directly.

If it is assumed that the model presented here is valid the following implications of relevance to radiological protection may be inferred: The equations derived in the model are strictly non-linear but would provide an analysis of the results of high dose, high dose-rate radiobiological experiments to obtain values of coefficients which are applicable at low dose and low dose rate and which are of direct relevance to radiological protection. The equations would indicate that there is no threshold in the radiation effect and that at low doses a 'linear model' can be considered as applicable. The two parameters which would be of most importance for radiological protection are the absolute values of the 'α' coefficients and the values of the limiting relative biological efficiency. The 'α' coefficients would vary from cell to cell, from cell phase to cell phase and would be dependent to some extent on the environmental circumstances during and after the irradiation. The limiting relative biological efficiency would be dependent on the standard radiation, the cell type and the environmental circumstances but it would be somewhat higher than the RBE normally derived from radiobiological experiments. It would, in any case, be a constant value independent of dose and dose rate under standard conditions. A consequence of the model and the common mechanism would be that the limiting relative biological efficiency (RBE_0) would be the same for the different biological end-points in the same cell irradiated under the same circumstances.

The model would indicate that considerable care should be taken in the choice of examples which are used to estimate risks. Although the theory would provide equations to describe biological end-points which could be used to extrapolate to low dose, low dose-rate conditions the equations would only apply to a homogeneous population irradiated under controlled conditions of uniform dose rate and radiation quality. These are conditions which are not usually satisfied in the cases used for the estimation of risks, at least somatic risks.

The molecular mechanism involved in the model is assumed to be common to all eukaryotic cells and as has been shown here can be related to many biological end-points. There would thus be a common basis for the extrapolation of results found in insects and animals to man. This extrapolation would of course have its restrictions and difficulties but at least the same fundamental process would be involved.

According to the model the molecular mechanism of DNA double strand breakage could form a common link between the biochemistry of DNA, and the radiation effect; the cell metabolism and how it affects the radiation effect via repair processes; the cell cycle and DNA configuration in the cell and the radiation effect; and it may provide a means of interpreting the results of radiobiological and radiation genetics experiments for extrapolation to radiological protection.

It is interesting to speculate on one other point which would arise if the model is valid and that is, that the molecular process, which is assumed in the model to lead to the radiation effect, is a process which is not unique to ionizing radiation. The same process of DNA double strand breakage can be caused by viruses, chemical agents, ultrasonic radiation and ultra-violet light. Thus, the model would suggest that the radiation hazard is not unique or especially different than the other everyday hazards we face.

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