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ON THE INTERPRETATION OF THE PARAMETERS OF THE QUADRATIC MODEL FOR CELL SURVIVAL AFTER IRRADIATION

by

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ON THE INTERPRETATION OF THE PARAMETERS OF THE QUADRATIC MODEL FOR CELL SURVIVAL AFTER IRRADIATION

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Summary

A model for cell survival after irradiation is derived based on the assumption that the damage leading to cell death is either a DNA double-strand break or a DNA single-strand break, and that the DNA double-strand break can be produced either by the same ionizing particle of radiation, or by two independent ionizing particles. This model is approximated to the quadratic model for cell survival $S/S_o = e^{-\alpha D-\beta D^2}$, but a new radiobiological interpretation is given to the parameters. This interpretation is shown to be consistent with experimental data on the effects of oxygen and nitrogen postirradiation treatments on the mortality of drosophila eggs.

1. Introduction

Several models have been proposed in radiation biology to relate survival with dose of radiation. The ones that have been most

Key Words: Biological effect of radiation; Multitarget model; Two-component model; Quadratic model; Survival curves; Repair mechanism.

The quadratic model for cell survival was derived in three different ways by Kellerer and Rossi (1972), Chadwick and Leenhouts (1973), Douglas and Fowler (1976), and presented again with some corrections by Leenhouts and Chadwick (1978). Although the three derivations are based on different arguments, they produce the same interpretation for the parameters, which is as follows;

α: induction of double-strand breaks in the DNA produced by the same ionizing particle, and

β: induction of double-strand breaks in the DNA produced by two independent ionizing particles, one in each strand.

Therefore the basic assumption of this model is that the only damage leading to cell death is the DNA double-strand break. This assumption contradicts a considerable amount of evidence which says that cell killing may be caused by two independent processes, of which the first requires single-strand breaks, and the second requires double-strand breaks (Bender and Wolff, 1961; Bender and Gooch, 1962; and Tym and Todd, 1964).

In this paper we derive an expression for cell survival in which we consider that cell killing is caused by the two independent processes mentioned above, and we also consider that double-strand breaks can be produced either by a single ionizing particle or by two independent ionizing particles. This new model can also be approximated to the quadratic model, but the parameters have a different interpretation from that given above. Following, by using data presented in

Sankaranarayanan (1969) and Sankaranarayanan and Volkers (1980), we show that the usual interpretation given to the parameters of the quadratic model is not consistent with the evidence that the repair of double-strand breaks is less efficient than that of single-strand breaks (Resnick and Martin, 1976). However the interpretation given in this paper is consistent. Finally we show that under certain conditions the quadratic model derived here can be used for other biological effects.

2. A Model of Cell Survival

The survival model derived in this paper is based on the following assumptions:

(a) Within the nucleus of the cell there are N sites such that when they are damaged can cause cell death.

(b) In each site the damage leading to cell death is either a DNA double-strand break or a DNA single-strand break.

(c) Although a small number of strand breaks may arise from direct action of ionizing particles on the DNA molecule itself, the large majority of strand breaks arises from indirect action through water radicals which are created by the radiation in the close surroundings of the DNA molecule.

(d) The DNA double-strand break can be produced by two modes of radiation action;

(i) both strands are broken by the passage of one ionizing particle very close to the DNA and

(ii) the double strand is broken by the action of two independent ionizing particles passing close to the DNA.

(e) The number of ionizing particles that passes close to a nucleotide, which may or may not produce strand breaks in the DNA, follows a Poisson distribution with mean ωD, where D is the dose of radiation and ω is the probability by unit of dose that an ionizing particle passes close to the DNA.

Let k be the probability that an ionizing particle passing close to a nucleotide leads to strand breakage. This parameter depends on the radiation quality and on the chemical situation in the nucleus of the cell (water content, etc.).

Let Ω be the probability that when the ionizing particle passing close to the first DNA strand it also passes close to the second one. This parameter depends on the radiation quality and also on the geometry of the DNA molecule.

Therefore after dose D, the mean number of ionizing particles that passes close to one strand without passing close to the other one is $\omega D(1 - \Omega)$, and the mean number of ionizing particles that passes close to both strands is $\omega D\Omega$. By using Poisson distribution, the probability of having x ionizing particles close to the "first" strand only, y ionizing particles close to the "second" strand only, and z ionizing particles close to both strands is given by

$$P_{x,y,z} = \frac{e^{-2\omega D(1-\Omega)}(\omega D(1-\Omega))^{x+y} e^{-\omega D\Omega}(\omega D\Omega)^{z}}{x!\ y!\ z!} .$$

If, after dose D, P_2 is the probability per site of double-strand break and P_1 is the probability per site of single-strand break we have

$$P_2 = \sum_{x=0}^{\infty} \sum_{y=0}^{\infty} \sum_{z=0}^{\infty} P_{x,y,z}[1 - (1-k)^{x+z}][1 - (1-k)^{y+z}]$$

and

$$P_1 = 2 \sum_{x=0}^{\infty} \sum_{y=0}^{\infty} \sum_{z=0}^{\infty} P_{x,y,z}[1 - (1-k)^{x+z}][(1-k)^{y+z}] ,$$

where $P_{x,y,z}$ is given above. The resulting expressions are

$$P_2 = 1 - e^{-k^2\omega D\Omega} + e^{-k^2\omega D\Omega}(1 - e^{-k\omega D(1-k\Omega)})^2 \qquad (1)$$

and

$$P_1 = 2(e^{-k\omega D} - e^{-2k\omega D + k^2\omega D\Omega}) . \qquad (2)$$

Expression (1) is the same as that found by Neary (1965) for chromosome aberrations. However the parameters here have a different interpretation because Neary (1965) has used assumptions of the Target Theory which are based on the direct action of radiation on the DNA.

Let f_o be the proportion of DNA double-strand breaks which are not repaired, let f_1 be the proportion of DNA single-strand breaks which are not repaired before a second single-strand break converts it

to a double-strand break, and let f_1^* be the proportion of DNA single-strand breaks related to cell death which are not repaired. Therefore, by introducing the parameters f_o, f_1 and f_1^* in expressions (1) and (2), the mean number of sites with either double-strand breaks or single-strand breaks which are not repaired is given by

$$N[f_o(1 - e^{-k^2\omega D\Omega}) + f_o f_1 e^{-k^2\omega D\Omega}(1 - e^{-k\omega D(1-k\Omega)})^2 + 2f_1^*(e^{-k\omega D} - e^{-2k\omega D+k^2\omega D\Omega})] \tag{3}$$

Let $\underline{p_1}$ be the probability that cell death is caused by single-strand breaks and $\underline{p_2}$ be the probability that cell death is caused by double-strand breaks. Hence the mean number of sites that causes cell death is given by

$$\mu = N[p_2 f_o(1 - e^{-k^2\omega D\Omega}) + p_2 f_o f_1 e^{-k^2\omega D\Omega}(1 - e^{-k\omega D(1-k\Omega)})^2 + 2p_1 f_1^*(e^{-k\omega D} - e^{-2k\omega D+k^2\omega D\Omega})] \quad . \tag{4}$$

By assuming that the number of sites that causes cell death follows a Poisson distribution with mean μ given above we obtain the following expression for cell survival

$$S/S_o = e^{-\mu} \quad . \tag{5}$$

For $k\omega D$ small, expression (4) can be approximated by taking the lowest order terms in the expansion of the exponentials and it

reduces to

$$\bar{\mu} \cong N[p_2 f_o k^2 \omega D\Omega + p_2 f_o f_1 (1 - k^2 \omega D\Omega)(k\omega D(1 - k\Omega))^2$$

$$+ 2p_1 f_1^* ((1 - k\omega D) - (1 - 2k\omega D + k^2 \omega D\Omega))] \quad .$$

Therefore survival expression (5) can be approximated to the quadratic model

$$S/S_o = e^{-\alpha D - \beta D^2} \quad , \qquad (6)$$

where

$$\alpha = N(2p_1 f_1^* k\omega(1 - k\Omega) + p_2 f_o k^2 \omega\Omega)$$

and

$$\beta = N(p_2 f_o f_1 k^2 \omega^2 (1 - k\Omega)^2) \quad .$$

The first term in α can be split into two parts as follows

$$2p_1 f_1^* k\omega(1 - \Omega) + 2p_1 f_1^* k(1 - k)\omega\Omega \quad .$$

The first part is the probability by unit of dose that an ionizing particle passing near a single DNA strand breaks it, and this single strand break is not repaired and causes cell death. The second part is the probability by unit of dose that the same ionizing particle passing near both DNA strands breaks one but does not break the other one, and this single-strand break is not repaired and causes cell death. The term $p_2 f_o k^2 \omega\Omega$ in α is the probability by unit of dose that a ionizing particle passing near both DNA strands produces

a double-strand break, and this double-strand break is not repaired and causes cell death. Therefore the parameter α gives the contribution to cell death of single-strand breaks and of double-strand breaks produced by mode (i) of radiation action (both breaks by the same ionizing particle).

In the same way we can see that the parameter β gives the contribution to cell death of double-strand breaks produced by two independent ionizing particles.

3. Discussion

The parameters p_1, which relates single-strand breaks to cell death and p_2, which relates double-stand breaks to cell death, depend on the cell itself and on the biological system under study. Different irradiation conditions should not reflect any changes in these parameters. If for some experimental situation one can assume that $p_1 = 0$, that is, the only damage related to cell death is the double-strand break, the parameters α and β will have the same structure as that given by Leenhouts and Chadwick (1978). If, on the other hand, $p_2 = 0$ model (6) will reduce to the exponential model

$$S/S_o = e^{-2p_1 f_1^* k\omega(1-k\Omega)ND} .$$

The parameter Ω, as we have mentioned before, depends on the radiation quality. For very low LET radiation ($\Omega = 0$) the parameter

α is still different from zero because it contains the contribution of the single-strand break and survival curve (6) still has a slope different from zero at very low dose. This information is very important for radiation protection.

The parameters f_o, f_1 and f_1^* are related to the repair mechanism, and therefore depend on the metabolic activity of the cell, the cell stage and the time available for repair. As we have mentioned before there is evidence that the repair of double-strand breaks is less efficient than that of single-strand breaks, hence we expect $f_o > f_1$ and $f_o > f_1^*$. Although the parameters f_1 and f_1^* are related to single-strand breaks repair, we have assumed that they may have different values, because according to their definition they are different in essence. The parameters f_o, f_1 and f_1^* are very important to explain the variation in the survival probability obtained from dose-fractionation regimes or from effects of postirradiation treatments.

In the dose-fractionation studies a dose D is given in a certain number of fractions with a period of time between them. This increases the repair capacity, and as a consequence the survival increases (see Figure 1). By looking at expression (6), we may have an increase in

(Place Figure 1 Here)

survival by decreasing either α or β, or also by decreasing both α and β. Table 1 shows the estimates of α and β based on data pre-

(Place Table 1 Here)

sented in Sankaranarayanan and Volkers (1980).

The estimates of the parameters were obtained by the non-linear least-squares procedure. Since the response variable (proportion of eggs hatched) is of the binomial type we have fitted the model by weighted and unweighted non-linear regression. The estimates and their variances obtained by unweighted and weighted regression are essentially equal. We present only the estimates obtained by unweighted non-linear regression using the Marquardt iterative method in double precision. By examining table 1 we conclude that both parameters α and β decrease with fractionation and the influence in α seems to be more effective than in β. This can be explained by the presence of the parameter f_1^* in α, different from f_1 in β. We would like to point out that in the derivation of the quadratic model given by Leenhouts and Chadwick (1978) the parameter α does not contain any parameter related to DNA single-strand breaks and therefore they conclude that we should expect the influence of dose fractionation be more effective in β than in α. This statement is not consistent with the results in Table 1.

In the postirradiation effect the comparison of several treatments is made through the changing in survival (see Figure 2, 3 and 4). According to

(Place Figures 2, 3 and 4, Here)

the discussion presented in Leenhouts and Chadwick (1978), the post-irradiation modification of the biological effect reflects a modification in the number of damaged sites. Then, under the assumption that the only damage related to cell death is the DNA double-strand break, the influence of postirradiation treatments on the parameters α and β would be through f_o. Therefore the parameters α and β should vary in the same proportion when we compare two postirradiation treatments (Leenhouts and Chadwick, 1978). However Table 2 shows that estimates

(Place Table 2 Here)

of the parameters α and β, based on data on the effects of oxygen and nitrogen postirradiation treatments presented in Sankaranarayanan (1969), are not consistent with the statement above. On the contrary, Table 2 shows that when we compare nitrogen and oxygen posttreatments in the first two situations and nitrogen and air in the third one, the variation in α is more significant than in β. Again this can be explained by the presence of the parameter f_1^* in α if we assume that the damage related to cell death can be either DNA double-strand breaks or DNA single-strand breaks. Here the estimates of the parameters were obtained by the same method as that of table 1.

4. Conclusions

In the derivation of survival models presented here, one can distinquish two sets of parameters. The first one is composed of ω, k, f_o, f_1, f_1^*, Ω and N which are parameters related to the production

of DNA double-strand breaks and DNA single-strand breaks that may or may not cause cell death, that is, parameters related to the potential damage produced by radiation action. The second set is composed of p_1 and p_2, parameters which relate the potential damage to the final biological effect (in this case death of the cell). Hence model (6) may be used for any other biological effects if we can assume that it is produced from the same potential damage (DNA double- or single-strand breaks). Thus, to generalize model (6) in order to be applied to several biological effects, we have to correct the definition of p_1 and p_2 by saying that the parameters p_1 and p_2, besides depending on cell type and biological system, depend also on the biological effect under study.

Sometimes it is convenient to express the model in terms of the yield of the effect instead of survival. For instance, suppose that $Y(D)$ is the expected probability of cancer of dose D corrected by the control for certain types of cells, and suppose that the cell damage related to production of cancer is either DNA double-strand breaks or DNA single-strand breaks. Then from expression (6) we obtain

$$Y(D) = 1 - e^{-\alpha D - \beta D^2} , \qquad (7)$$

where α and β are given in (6). Model (7) is the two-stage model (Armitage and Doll, 1961). Our derivation takes in account the action of radiation on the cell and therefore may give us a better interpretation of the results.

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Table 1

Estimated changes in the parameters of $S/S_o = e^{-\alpha D-\beta D^2}$ with different radiation regimes. (The model was fitted to data presented by Sankaranarayanan and Volkers, 1980).

Radiation Regimes	Nonlinear Least Square Estimates	
	α	β
Unfractionated	.1075 $\pm$ .0130	.0470 $\pm$ .0043
Two Fractions	.0625 $\pm$ .0119	.0374 $\pm$ .0033
Three Fractions	.0371 $\pm$ .0193	.0308 $\pm$ .0050

Table 2

Estimated changes in the parameters of $S/S_o = e^{-\alpha D-\beta D^2}$ with different postirradiation treatments and different initial conditions. (Data from Sankaranarayanan, 1969).

Experimental Conditions	Nonlinear Least Square Estimates	
	α	β
N_2 - R - N_2	.0964 ± .0111	.0092 ± .0017
N_2 - R - O_2	.0452 ± .0092	.0094 ± .0012
O_2 - R - N_2	.2062 ± .0201	.0764 ± .0079
O_2 - R - O_2	.1081 ± .0174	.0619 ± .0059
Air - R - N_2	.1666 ± .0086	.0335 ± .0023
Air - R - Air	.0891 ± .0107	.0273 ± .0024

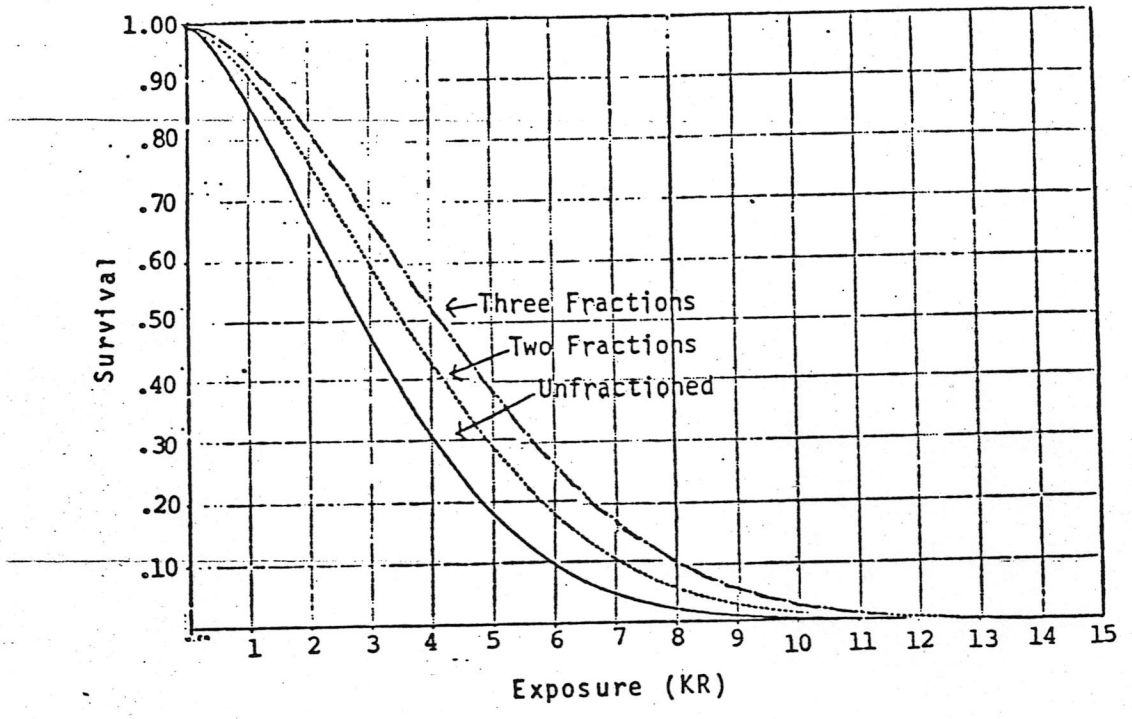

Fig. 1.- Relationship between X-ray exposures and survival of eggs irradiated as stage-7 oocytes after acute and fractionated irradiation. The curves represents the quadratic model $S/S_0 = e^{-\alpha D - \beta D^2}$ fitted to data presented by Sankaranarayanan and Volkers, 1980.

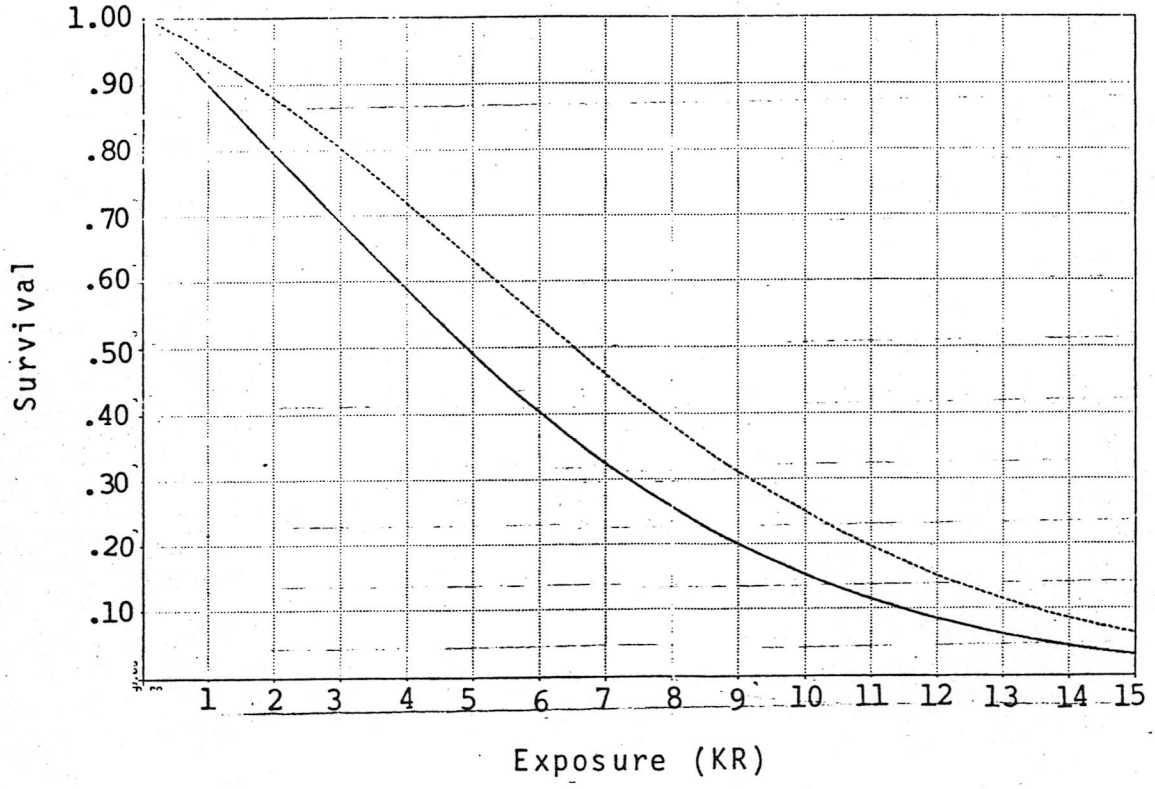

Fig. 2 - Survival of stage-7 oocytes after irradiation in N_2 followed by either N_2 or O_2 pos-treatment. The curves represents the quadratic model $S/S_o = e^{-\alpha D - \beta D^2}$ fitted to data presented by Sankaranarayanan, 1969.

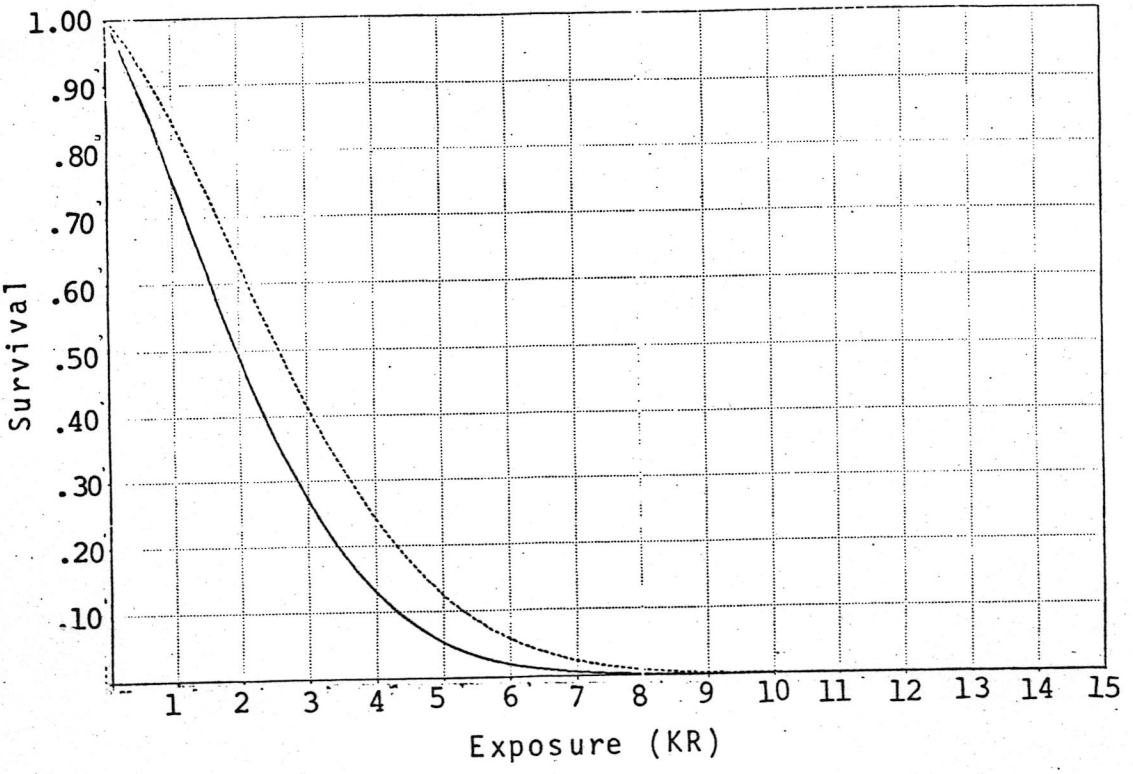

Fig. 3 - Survival of stage-7 oocytes after irradiation in O_2 followed by either N_2 or O_2 pos-treatment. The curves represents the quadratic model $S/S_o = e^{-\alpha D - \beta D^2}$ fitted to data presented by Sankaranarayanan, 1969.

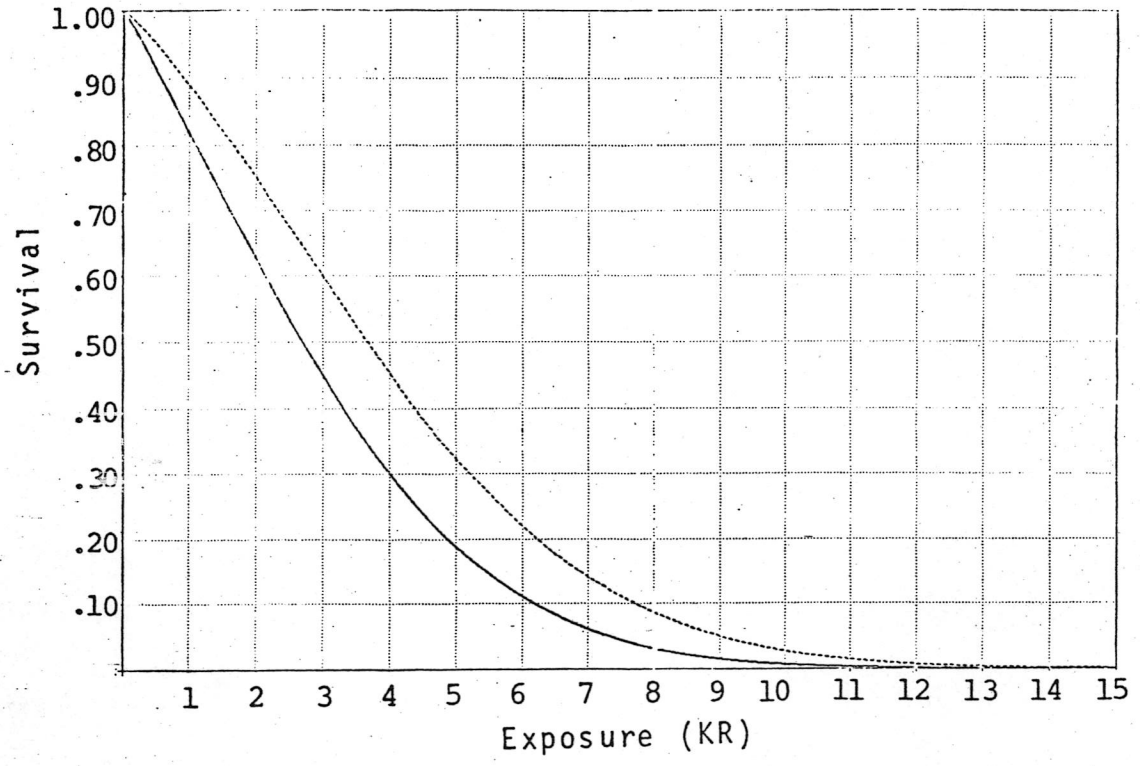

Fig. 4 - Survival of stage-7 oocytes after irradiation in Air followed by either N_2 or Air pos-treatment. The curves represents the quadratic model $S/S_o = e^{-\alpha D - \beta D^2}$ fitted to data presented by Sankaranarayanan, 1969.

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