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Empirical Evaluation of Cell Critical Volume Dose Vs. Cell Response Function for Pink Mutations in Tradescantia

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Empirical Evaluation of Cell Critical Volume Dose Vs. Cell Response Function for Pink Mutations in Tradescantia[1]

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Abstract

Microdosimetric spectra for 0.43, 1.8, and 14.7 MeV neutrons, and for 215 kVp x rays and 1250 keV gammas were used in conjunction with relative biological effectiveness (RBE) values for pink mutations in Tradescantia to obtain an effectiveness function (i.e., a cell critical volume dose vs. cell response function). This effectiveness function (or hit size weighting function) provides the probability of inducing a biological effect of interest (in the present study, pink mutations in Tradescantia) as a function of lineal energy density y. In a preliminary analysis the critical value of y above which pink mutations are seen was 4.5 keV/μm, and the value of y at which the probability reaches unity was 115 keV/μm. Idealized but approximate event size distributions for mono-LET particles ranging from 10 to 5000 keV/μm were generated, and these distributions were weighted by the effectiveness function to determine the pink mutation frequencies. Results are compared with measured pink mutation frequencies for 11 keV/μm (^{12}C) and 31 keV/μm (^{20}Ne) ions.

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Introduction

In order to explain the radiobiological dose response curves for cell killing, mutations, chromosome abnormalities, or malignant transformations, numerous models, theories, and hypotheses have been advanced (1-5). However, these models do not include a threshold concept and are based on combinations of linear and quadratic components in the dose-response function. There is evidence that the quadratic component, observed prominently in low-LET dose-response curves, is seen primarily at high doses and dose rates, which do not allow time for the repair of subeffect damage and thus mask the linear component. A number of experiments show that if the dose is low, or is given over a period of time sufficiently long to allow repair processes to be operative, the quadratic component of the dose-effect curve is essentially eliminated (6-7).

The analysis that follows is strictly limited to that portion of the dose-response curve, where the response is linear. Bond (8) has suggested that the usual form of dose-response curve is incorrectly interpreted at low doses where single-hit kinetics predominate the effect. This is obvious when one considers that at low dose very few cells in an exposed population of cells get any energy deposit. In practice, however, the absorbed dose is calculated and this is interpreted to mean that each exposed cell receives some average energy deposit. This picture leads to misinterpretation of dose-response curves. Bond and Varma (9) have proposed that to be consistent with the vast majority of dose-response curves obtained in pharmacology, one must obtain a similar function for ionizing radiations at the cellular level.

This function, which is termed the effectiveness function, represents the probability of an all-or-none effect as a function of hit size (which is equivalent to size of energy deposit, ε). This function, in principle, can be obtained by combining a vast amount of radiobiological data at low dose and microdosimetric data for different radiation qualities for which radiobiological data are available. The formulation and conceptual importance of this effectiveness function have been described in detail (9). This paper describes details on the method for evaluating the effectiveness function for one particular end point, pink mutations in Tradescantia.

Experimental

a. Microdosimetric data

A Rossi-type proportional counter is placed in the field of ionizing radiation, and the count rate is adjusted either by varying the position of the detector with respect to the source, or the strength of the source, such that the counter records only single event spectra. Details on the microdosimetric technique and counter are available elsewhere (10), and will not be discussed here. In the present study, event size distributions in a one micron simulated site have been used for 0.43 and 14.7 MeV monoenergetic neutrons. For 1.8 MeV neutrons event size distributions a 1.3 micron simulated site was used. The distributions for 0.43 and 1.8 MeV neutrons were obtained from Rosenzweig et al. (11), and for 14.7 MeV neutrons from Biavati et al. (12). For 215 kVp x rays and 1250 keV gammas (from ^{60}Co) event size distributions in a one micron site, measured by Varma et al. (13), were used.

b. Radiobiological data

A considerable amount of data on pink mutations in Tradescantia at low doses for a variety of neutron energies, x rays, and ^{137}Cs and ^{60}Co gamma rays are available. Details on the experiments, method of scoring, and results have been published (6,7,14). These data show that at low doses the incidence of pink mutations is linearly dependent on absorbed dose. Also, it has been shown (6,7) that the slope of the low-dose portion of the low-LET curve is also obtained with higher doses provided that the dose rate is low enough to permit essentially full repair of subeffect damage. Thus, Tradescantia data are ideal for evaluating the effectiveness function using single event microdosimetric data as mentioned above. In this study, pink mutation data on 0.43, 1.8, and 14.7 MeV neutrons, and 250 keV x ray and 1250 keV gammas, available in the literature (6,7,14), were used to determine RBE values for different neutron energies and 1250 keV gammas with respect to 250 kVp x rays.

c. Evaluation of effectiveness function

Consider first for a given absorbed dose D a distribution of the number of events N(y) as a function of lineal energy density y, where y is defined as the ratio of energy deposited (in a volume simulating a site of diameter d) and the mean chord length $\bar{\ell}$. If n(y) denotes the number of events per unit dose then n(y) can be obtained from an N(y) distribution as follows:

$$n(y) = \frac{N(y)}{\int N(y) \cdot y dy} \frac{d^2}{20.4} \qquad (1)$$

where d is in microns and y is in keV/micron. The numerical factor 20.4 converts the units from keV/μm and μm to rads.

Let E(y) represent the effectiveness function which is the probability of an effect for a given lineal energy density y. In accordance with the vast majority of dose-response curves in pharmacology, it is assumed that at some critical value of energy deposit ε_o or lineal energy density y_o, cells will begin to express the end effect of interest. The probability of expressing the effect will increase as the y value increases and reach unity at some sufficiently high value of y, say y_m. The exact functional form between y_o and y_m is not defined. For the present preliminary analysis, we have assumed a functional form for E(y) given in equation (2).

$$\begin{aligned}
E(y) &= 0 && y_o \geq y \\
E(y) &= \frac{y-y_o}{\alpha-y_o} && y_o \leq y \leq y_1 \\
E(y) &= \left(1-\frac{y_1-y_o}{\alpha-y_o}\right)\frac{y-y_1}{y_m-y_1} + \frac{y_1-y_o}{\alpha-y_o} && y_1 \leq y \leq y_m \qquad (2) \\
E(y) &= 1 && y \geq y_m
\end{aligned}$$

where α, y_o, y_1, and y_m are constants.

The product of the effectiveness function E(y) and the number of events per unit dose n(y) gives the fraction of cells that will show an effect per rad at a given y. The total fraction of cells showing the effect per rad is

obtained by summing the above product for all possible values of y. Let f_i represent the fraction of cells showing the effect per rad for a given quality of radiation i, and corresponding number of events per rad by n_i, then

$$f_i = \int n_i\,(y)\; E\,(y).\, dy \qquad (3)$$

Let the ratio of effects due to a radiation of quality i, and that of a standard radiation (normally 250 kVp x rays), be represented by R_i, then

$$R_i = \frac{\int n_i\,(y)\; E\,(y)\; dy}{\int n_s\,(y)\; E\,(y)\; dy} \qquad (4)$$

where n_s is the number of event per rad for the standard radiation.

Since we are in the domain of the linear portion of the dose response curve, RBE which is defined as the ratio of doses for standard and test radiations for a given level of effect, is identically equal to the ratio of effect per unit dose due to test and standard radiations. Thus, R_i in equation 4 is equivalent to RBE.

In the present study we have evaluated R_i by using microdosimetric data for 0.43, 1.8, and 14.7 MeV neutrons, and for 1250 keV gammas. The standard radiation for this evaluation was 215 kVp x rays. By trial and error, parameters y_o, y_1, α, and y_m were varied to obtain a reasonable agreement (within a factor of 2) between R_i and the RBE values for pink mutations in Tradescantia. Values obtained for y_o, y_1, α, and y_m were 4.5, 68, 500, and 115 keV/μm, respectively. Using these parameters, the following functional dependence of E(y) was obtained:

$$\begin{aligned}
E(y) &= 0 && y \leq 4.5 \\
E(y) &= 2.02 \times 10^{-3}\, y - 9.08 \times 10^{-3} && 4.5 \leq y \leq 68 \\
E(y) &= 1.855 \times 10^{-2}\, y - 1.133 && 68 \leq y \leq 115 \\
E(y) &= 1 && y \geq 115
\end{aligned} \qquad (5)$$

Results and Discussions

Using the effectiveness function given in equation (5) and the microdosimetric spectra for 215 kVp x rays (in one micron site diameter) and equation (3), the number of pink mutations per cell per rad was at 1.0×10^{-5}. Table 1 lists calculated and measured values of R_i and the absolute number of pink mutations per cell per rad for 14.7, 1.8, and 0.43 MeV neutrons, and for ^{60}Co gammas and 215 kVp x rays.

Table 1

Radiation	R_i		f_i	
	Observed	Calculated*	Observed	Calculated*
0.43 MeV neutron	46	23.5	1.1×10^{-2}	2.35×10^{-4}
1.8 MeV neutron	16	15.5	3.9×10^{-3}	1.55×10^{-4}
14.7 MeV neutron	10	11.0	2.4×10^{-3}	1.1×10^{-4}
1250 keV gammas	0.5	0.45	1.2×10^{-4}	4.5×10^{-6}
215 kVp x ray	1.0	1.0	2.4×10^{-4}	1.0×10^{-5}

*Based on one micron site diameter.

Figure 1 shows a plot of the effectiveness function represented by equation 5. Figure 2 shows a plot of pink mutation frequency in Tradescantia as a function of absorbed dose for 0.43 MeV neutrons and 250 kVp x rays. From this Figure, the absolute number of pink mutations per stamen hair per rad for 250 kVp x rays is seen to be 6×10^{-4}. It has been suggested (15) that each stamen hair has, on an average, about 2.5 cells at risk. Thus, the number of pink mutations per cell at risk per rad will be approximately 2.4×10^{-4}. The number of pink mutations per cell per rad determined from the present study was 1.0×10^{-5}. The difference in calculated and measured pink mutations is attributed to the fact that the site diameter of one micron used in our analysis may not be correct. However, since the number of events per unit dose scales approximately as the square of the site diameter 'd' the correct site diameter can be obtained by using the following equation.

$$d = \sqrt{\frac{f_i \text{ (observed)}}{f_i \text{ (calculated)}}} \qquad (6)$$

where f_i is the absolute number of pink mutations per cell per rad for 215 kVp x rays and d is in microns. Using equation (6) yields a value of 4.9 μm for the site diameter.

It should be emphasized that in the above calculation of the site diameter, it is assumed that event size distributions do not change between one and five microns. However, measured event size distributions in larger sites indicate that $\bar{y}_D$ which is the dose average lineal energy density changes by a factor of 2 for x rays. The influence of site size on the effectiveness function has to be evaluated to obtain a more precise value for the site diameter. The site diameter of 4.9 μm can also be viewed as the upper limit of the critical volume of the cell in which energy deposition must take place to express pink mutations and is approximately equal to the diameter of the cell nucleus in Tradescantia.

In order to augment the neutron, gamma, and x-ray results, similar calculations for a variety of charged particles having different LET's were made. However, since measured microdosimetric spectra for these particles were unavailable, idealized event size spectra were generated for use in the analysis. Thus, the results from this analysis were expected to provide qualitative and not quantitative information.

In track segment experiments charged particles of relatively well-defined LET are used. However, each monoLET particle still has a distribution in y values. Approximating a track as a line (i.e., radius << site diameter) the distribution of number of events as a function of y for these particles in spherical sites is triangular and can be expressed by the following equation.

$$N(y) = \alpha y \qquad 0 \leq y \leq \frac{3L}{2} \tag{7}$$

where N(y) is number of events at a given y per unit y for a given absorbed dose D and the maximum possible y-value for a given LET, L is 3L/2, and α is a constant. The number of events at a given y per unit y per unit dose can be obtained easily from the above equation as

$$n(y) = \frac{4}{3L^2} \frac{d^2}{20.4} y \tag{8}$$

where d is in μm, L is in keV/μm, and 20.4 is the numerical factor which con-

verts keV/μm and μm into rads. However, in real situations n(y) will not be triangular but may be distorted at low y values due to contributions from electrons and gamma rays which are present as contaminants in the charged particle beams.

To predict the pink mutations per cell per rad a set of triangular distributions represented by equation (7) were generated for LET values ranging from 10 to 5000 keV/μm. These distributions were weighted by the effectiveness function obtained for Tradescantia (eq. 5). Taking the ratio of pink mutation frequency for a given LET, with that of 215 kVp x rays (standard radiation) RBE values can be estimated. Table 2 presents the calculated pink mutations per cell per rad for different LET's along with the RBE values. Figure 3 shows the variation of pink mutation per cell per rad as a function of LET of the charged particle, on the right ordinate corresponding RBE values relative to x rays shown. From this plot it can be seen clearly that the RBE increases when LET reaches a maximum around 100 keV/μm and then decreases. This qualitative behavior for change in RBE as a function of LET has been observed in experimental studies of other biological systems (16).

From the above analysis the RBE's for 11 and 31 keV/μm monoLET particles were determined as 5.9 and 8.4, respectively. Measured values for 11 keV/μm (^{12}C ions) and 31 keV/μm (^{20}Ne ions) were 2 and 5, respectively (17). Comparison of measured and calculated RBE values for these two LET's shows that our analysis indicate about 3 and 1.7 times higher RBE values for 11 and 31 keV/μm, respectively. Higher values obtained in our calculations may be partially due to the presence of low LET components in the charged particle beams used for measurements of pink mutations in Tradescantia.

In conclusion, the method described in this paper, which is based on combining the microdosimetric and radiobiolgoical data to obtain the effectiveness function does work in principle. However, more work needs to be done to study the dependence of the effectiveness function on such parameters as site size, end effect of interest, and other biological systems. Presently, we are engaged in refining the functional form of the effectiveness function.

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Table 2

Calculated+ Pink Mutations Per Cell Per Rad,
and RBE's for Charged Particles with Various LET Values

LET	Pink mutations/cell/rad	RBE*
3	0	-
6	3.1×10^{-5}	3.1
10	5.4×10^{-5}	5.4
20	7.8×10^{-5}	7.8
30	8.3×10^{-5}	8.3
50	9.8×10^{-5}	9.8
70	2.2×10^{-4}	22.0
100	3.2×10^{-4}	32.0
200	2.2×10^{-4}	22.0
300	1.6×10^{-4}	16.0
500	9.6×10^{-5}	9.6
1000	4.8×10^{-5}	4.8
2000	1.6×10^{-5}	1.6
5000	9.8×10^{-6}	1.0

*RBE's expressed with respect to 215 kVp x rays

+Based on one micron site diameter

References

1. R. Katz, S. C. Sharma, and M. Homayoofor, Topics in Radiation Dosimetry (F. H. Attix, Ed., Academic Press, NY) 317 (1972).
2. D. E. Lea, Actions of Radiations on Living Cells (Cambridge University Press, London and NY) (1946).
3. A. M. Kellerer and H. H. Rossi, Current Topics in Rad. Res. Quart. 8, 85 (1972).
4. A. M. Kellerer, IAEA Technical Report Series No. 58 (International Energy Agency, Vienna) 95, (1966).
5. H. P. Leenhouts, K. H. Chadwick, Int. Radiat. Bio. 33, 357 (1978).
6. V. P. Bond, Radiat. Environ. Biophysics 17, 1 (1979).
7. NCRP Publication 64, Influence of dose and its distribution in time on dose response relationships for low LET radiation, National Council on Radiation Protection and Measurements, Washington, DC (1980).
8. V. P. Bond, The Conceptual Basis for Evaluating Risk From Low-Level Radiation Exposure, in the Proceedings of the 1981 Annual Symposium and Meeting of the NCRP (1981).
9. V. P. Bond and M. N. Varma, Low Level Radiation Response Explained in Terms of Fluence and Cell Critical Volume Dose, to be presented at the Eighth Symposium on Microdosimetry, September 27-October 1, Kernforschungsanlage, Julich (1982).
10. H. H. Rossi, Radiation Dosimetry, Vol. I (F. H. Attix, W. C. Roesch, Eds., Academic Press, NY) (1968).
11. W. Rosenzweig, H. H. Rossi, I. Miyana, and F. Bailey, Annual Report, Radiological Research Laboratory, Columbia University, NY, NYO-9205, 25-38 (1960).
12. M. H. Biavati, H. H. Rossi, A. Hutton, and E. Boer, Annual Report, Radiological Research Laboratory, Columbia University, NY, NYO-2740-1, 32-48 (1964).

13. M. N. Varma, J. W. Baum, P. Kliauga, V. P. Bond, Microdosimetric Measurements For Photons in a Water Phantom, in Proceedings of the Seventh Symposium on Microdosimetry, EUR 7147 de-en-fr 775 (1980).

14. A. H. Sparrow, A. G. Underbrink, and H. H. Rossi, Science 176, 916 (1972).

15. L. Schairer, private communication (1981).

16. P. W. Todd, Radiat. Res. Suppl. 7, 196 (1967).

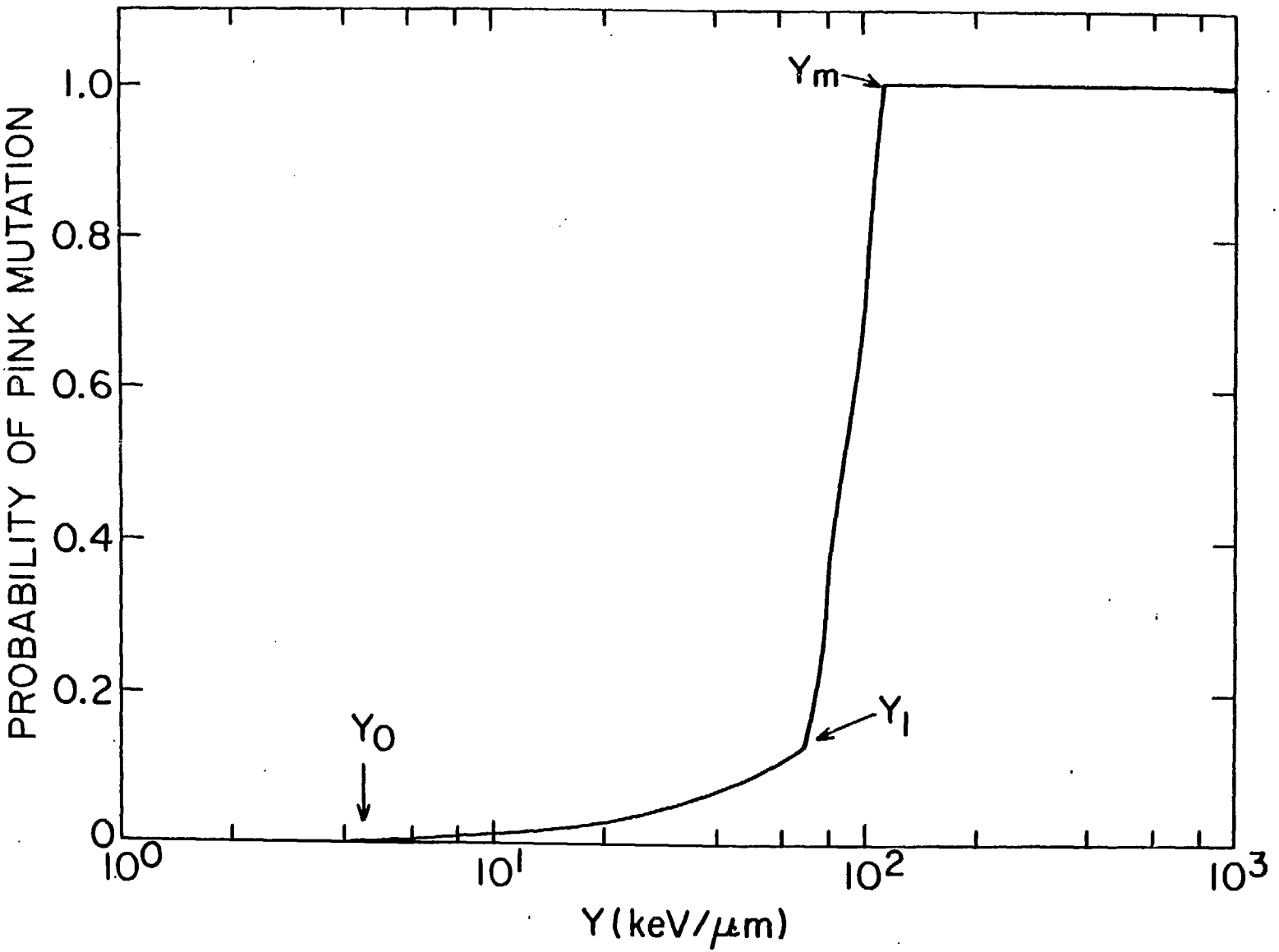
17. J. W. Baum, L. A. Schairer, and M. N. Varma, Physical Factors Influencing RBE in Production of Pink Mutations in Tradescantia Stamen Hairs, Brookhaven National Laboratory Report No. 25225 (1979).

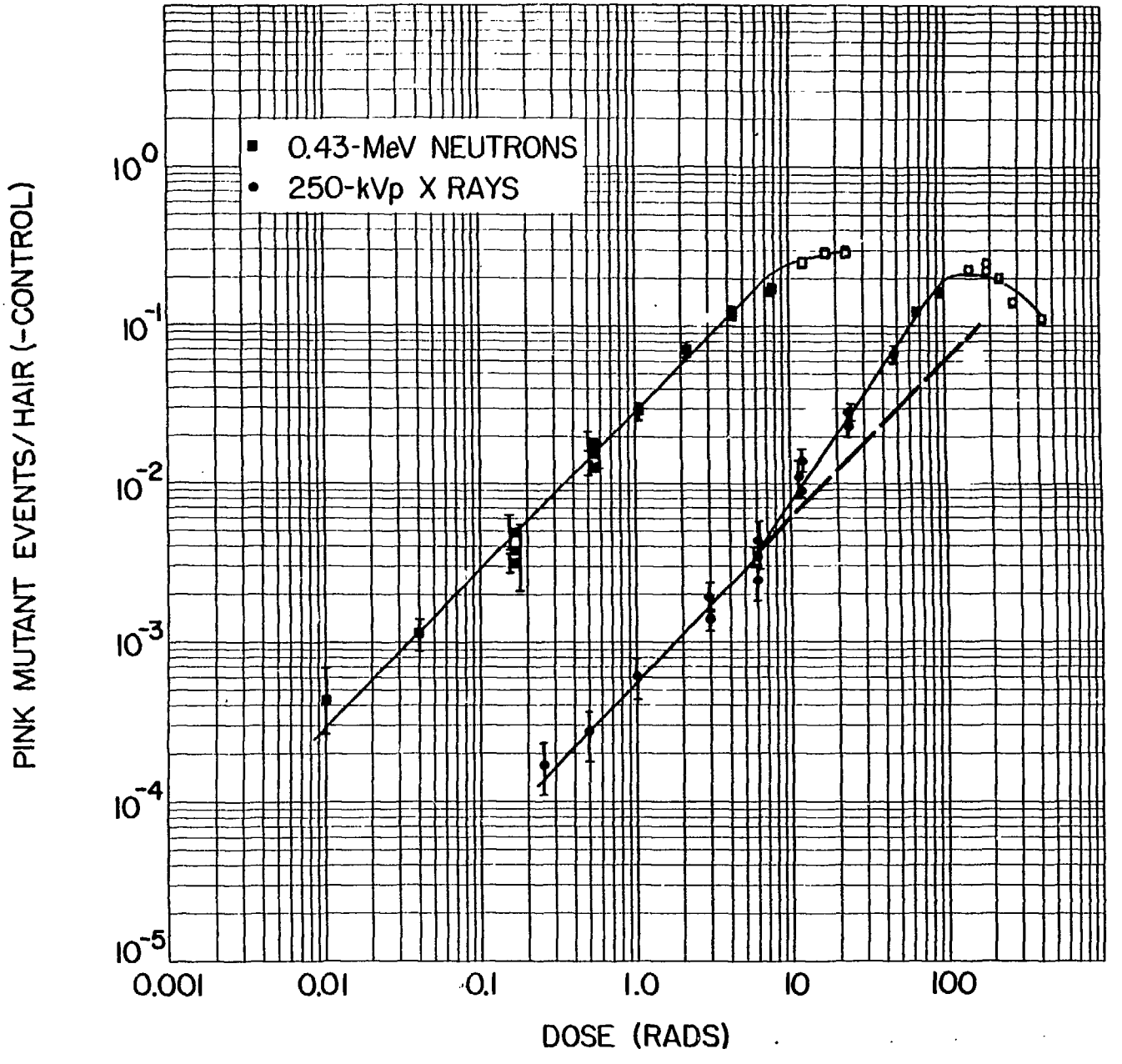
Figure Captions

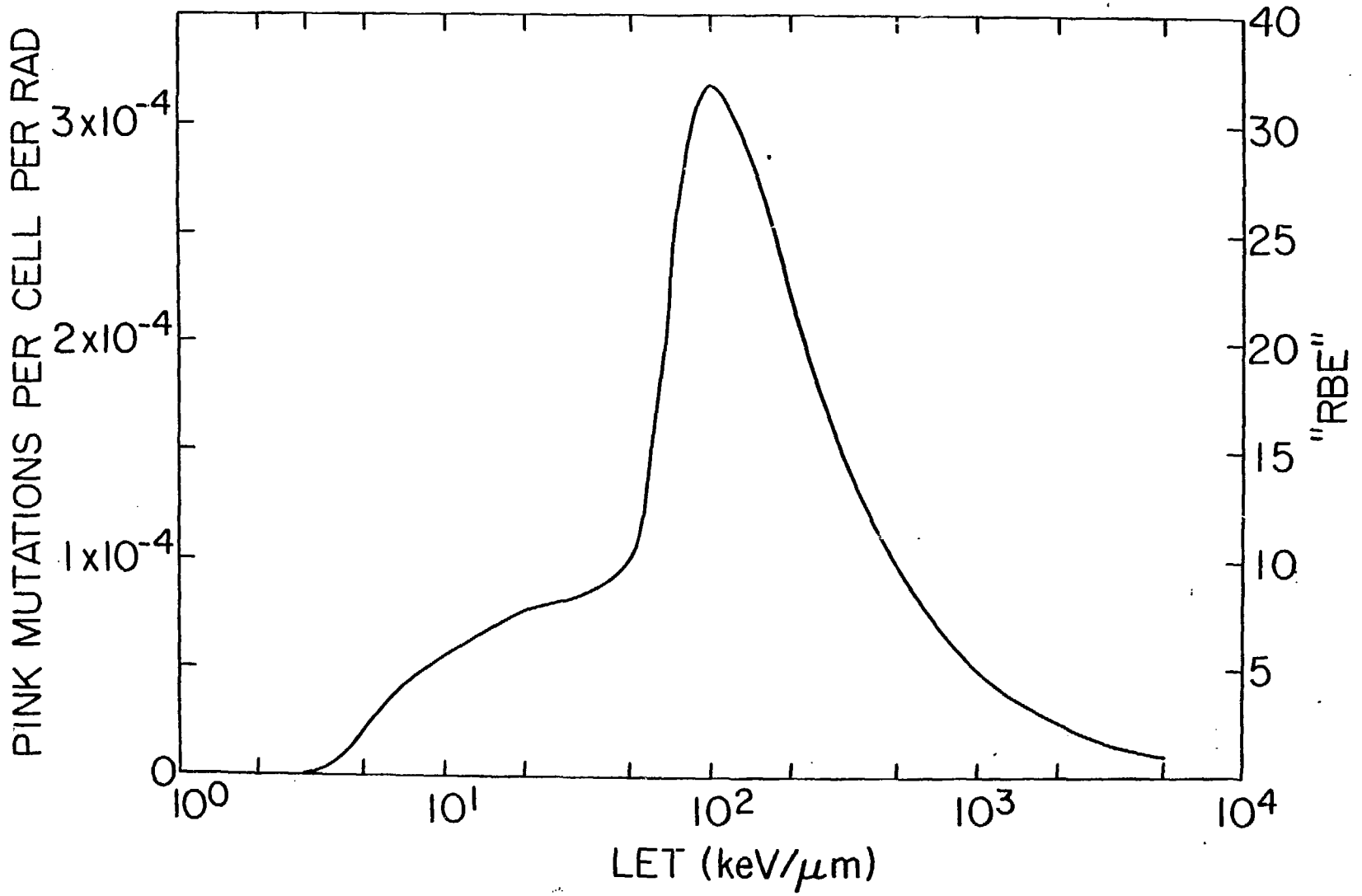
Figure 1 - Effectiveness function or probability of inducing a pink mutation as a function of Y.

Figure 2 - Experimentally measured pink mutations per hair (less control) for 250 kVp x rays and 0.43 MeV neutrons as a function of dose.

Figure 3 - Calculated pink mutations per cell per rad and RBE as a function of charged particle LET (for one micron site diameter).


PROBABILITY OF PINK MUTATION
1.0
0.8
0.6
0.4
0.2
0
Ym
Y1
Y0
10^0
10^1
10^2
10^3
Y (keV/μm)


0.43-MeV NEUTRONS
250-kVp X RAYS
PINK MUTANT EVENTS/HAIR (-CONTROL)
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
0.001
0.01
0.1
1.0
10
100
DOSE (RADS)


PINK MUTATIONS PER CELL PER RAD
3x10-4
2x10-4
1x10-4
0
40
30
25
20
15
10
5
"RBE"
10^0
10^1
10^2
10^3
10^4
LET (keV/μm)