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THE INFLUENCE OF EXPERIMENTAL END POINTS, DOSE, DOSE RATE, NEUTRON ENERGY, NITROGEN IONS, HYPOXIA, CHROMOSOME VOLUME AND PLOIDY LEVEL ON RBE IN TRADESCANTIA STAMEN HAIRS AND POLLEN*

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INTRODUCTION

It is well known that relative biological effectiveness (RBE) is a complex quantity which can be influenced by several factors. These may be physical, biological or experimental [1-7]. The project upon which we are reporting was undertaken to examine as many of these modifying factors as possible within a single eukaryotic biological test system. The test system makes use of the stamen hairs found in Tradescantia sp. clone 02. In cases where it was not feasible to use stamen hairs, pollen abortion was an alternative end point for several other members of the family Commelinaceae.

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To date, only some of the factors which influence RBE have been or are being investigated. Therefore, this report should be considered a progress report and the conclusions subject to change as more data accumulate. It is hoped that these investigations are being carried out in such a fashion that they may be analyzed within the framework of a biophysical theory based on principles of microdosimetry.

The Tradescantia research was done at Brookhaven National Laboratory and forms part of a joint project with Professor H. H. Rossi at Columbia University. Besides factors influencing RBE we are particularly concerned with very low levels of ionizing radiation for which the stamen hair system is especially well suited because of its high radiosensitivity [8].

Aspects of RBE which we have been investigating and upon which we report herein include the following: experimental end points (types of somatic aberrations, mutations or loss of reproductive integrity), dose (with special emphasis on very low doses), dose rate, neutron energy, nitrogen ions, hypoxia (in relation to both neutron energy and very low doses of x rays), ploidy level and interphase chromosome volume. Interphase chromosome volume (ICV) is an indirect measure of chromosome size and is obtained by dividing the nuclear volume by the chromosome number [9,10]. ICV has been shown to be one of the most important variables in determining the radiosensitivity of plants, and for allowing useful predictions of expected radiosensitivities to be made [9-16]. It may also influence RBE among species.

Tradescantia sp. clone 02 as well as certain other members of the Commelinaceae family were chosen for these investigations because they have several unique features which make them particularly desirable for this type of radiobiological experimentation [for references see 17]. Four features seem most important: 1) The plant is heterozygous for flower color and the flower cells readily mutate from the normal blue to pink. 2) Tradescantia clone 02 is very radiosensitive. Its somatic cells have about the same range of sensitivity as mammalian cells for loss of reproductive integrity but may be more sensitive for somatic mutations. Indeed, as we will show, a significant yield of mutations has resulted from doses as low as 10 mrads of 0.43-MeV neutrons and 0.25 rads of x rays [8]. 3) While other eukaryotic systems may also mutate at these low doses, Tradescantia has a distinct advantage in that hundreds of thousands of stamen hairs can be scored easily and quickly. This allows the gathering of enough data from very low dose irradiations to be statistically significant. Each inflorescence produces a flower about every other day and each flower bears about 300 stamen hairs. Each stamen hair consists of a file of about 20 cells. 4) A relatively short experimental period is required. We routinely score for induced mutations during days 11 through 15 after irradiation. These days are used because they encompass the days showing the highest mutation frequency for pink and colorless mutations (Fig. 1).

MATERIALS AND METHODS

The methods for the proper maintenance of Tradescantia and the manner in which experiments are routinely handled and scored have been detailed elsewhere [17]. For this report, a brief, general description of handling should suffice. Any significant departures from the general procedure will be mentioned with the appropriate topic.

Cuttings of Tradescantia sp. clone 02 topped by a young inflorescence are selected on the basis of similar stage of inflorescence development. These are selected from stocks maintained in growth chambers under standard conditions, 18-hr day, 1650 ± 50 foot candles and temperatures of 68° F for day and 64° F for night [17]. Cuttings are about 4-6 inches long and are usually allowed to root for 10 days before experimentation. Throughout the course of the experimental period they are kept in aerated Hoagland's solution under standard conditions in a controlled environment growth chamber.

The neutron irradiations were done at the Radiological Research Accelerator Facility (RARAF) at Brookhaven National Laboratory. The neutron dosimetry followed the general approach adopted for monoenergetic neutron studies. Dosimetry was performed with materials having the atomic composition of human muscle tissue. It is estimated that the plant tissues were of sufficiently similar composition to keep dose uncertainties within about 10% [7,18,19]. Gamma-ray contribution to the total dose for most neutron energies was insufficient to change the results significantly. Dose rates were usually about 0.5 rads/min.

For x irradiations, the plants were placed on Masonite instead of aluminum and covered with 1/16-inch tissue-equivalent plastic. The machine was operated at 250-kVp and 30 mA with 1.59 mm Cu and 0.608 mm Al. Target to x-ray tube distance was 82 cm with an average dose rate of 30 rads/min. Dosimetry was done with a Victoreen dosimeter [7].

The data from all experiments were analyzed on a CDC 6600 computer by programs written by Mr. K. H. Thompson.

Scoring procedures for stamen hairs have been described in detail elsewhere [17]. Most of the data presented here were acquired by a simple scanning technique in which the stamen is only scored for pink mutations or possibly another one of the most commonly occurring phenotypic aberrations [17].

One may predict the number of stamen hairs to be scored for many radiobiological situations [17]. Figure 2 is a graph showing the appropriate numbers of stamen hairs to be scored for resulting standard errors to fall within 1, 5, 10 and 20% of the mean values. The graph is based on experimental data obtained during the routinely used 5-day scoring period (days 11 through 15 after irradiation).

The left-hand scale in Fig. 2 shows the total numbers of stamen hairs needed as well as total flowers (based on an average of 300 hairs per flower). The numbers of stamen hairs and flowers needed each day for the 5-day scoring period are given on the right-hand scale. X-ray dose and aberration frequency (pink events/hair) are shown along the abscissa. Numbers of stamen hairs to be scored can be less than the numbers indicated in Fig. 2 because of the relatively high RBE values expected from clone 02 for most radiations.

As shown in Fig. 2, a low dose point obviously is reached where such large amounts of material are required that experiments cease to be practical. However, with Tradescantia, one can use very low doses before experiments become unfeasible.

Besides loss of reproductive integrity, more than 25 combinations of somatic cellular changes (mutations and aberrations) may be studied. Except for the change in color from blue to pink it is not certain whether the other types of aberrations are genetic in nature [17].

RESULTS AND DISCUSSION

A. Experimental End Points and RBE

It is possible to construct typical survival curves from Tradescantia stamen hair data (Fig. 3). Stamen hairs having 12 or more cells are taken to represent survivors since 95% of the control population consists of hairs 12 or more cells in length [7,20]. Days 17-19 after irradiation yield the maximum percentage of stamen hair stunting [20]. The resulting survival curves are rather typical for both neutron and x irradiations. The shapes of these curves can be changed by using stamen hairs of different lengths (cell numbers) as survivors or changing the days postirradiation on which the flowers are scored. Survival curves can also be constructed using only populations of hairs which contain specific mutations or aberrations, or conversely, using only those hairs which apparently contain no mutation [20]. The curves in Fig. 3 were constructed using all hairs. RBE at 50% survival (170 rads x rays/17 rads neutrons) is about 10 [7], and drops to about 7 (165 rads x rays/24 rads neutrons) at D_0. These rather low values are significant, for much higher RBEs occur for nonlethal, or mutation end points. The RBEs for pollen abortion, another lethal end point also tend to be relatively low for Tradescantia [21].

RBE values also differ for the various kinds of somatic aberrations or mutations in stamen hairs of the same species. RBE values derived from eight of the most frequently encountered aberrant cell types determined from dose-response curves for 0.43-MeV neutrons and 250-kVp x rays are given in Table I [22]. Figure 4 shows dose-response curves for pink mutations which are characteristic for seven of the eight different cell types studied. Typically, the neutron curves have a slope of 1 (linear on a log-log presentation), while the x-ray curves are usually steeper except at very low doses. However, computed curves for our x-ray data have never reached a slope of 2 [8].

At the higher doses, a region of saturation is reached and the mutation frequency levels off and then declines [7,17,22]. The reason for this phenomenon is not resolved, but it could be due to site saturation on the chromosome, cell lethality, or both.

In Fig. 4, the mutant events per hair are plotted as the accumulated results scored over days 7 through 16 postirradiation. These cumulative values from which the RBEs in Table I were derived were employed for the sake of convenience since some types of aberrations occur with a frequency too low to give the desired yields using the standard scoring period of 5 days. Moreover, the slopes of the curves (and thus RBEs) are not noticeably changed by using cumulative data [17].

A different type of response is illustrated by the curves for the blue giant cell type (Fig. 5) [7,22]. For this cell type, the number of aberrant events per hair does not appear to saturate at the higher doses for either type of irradiation. Another interesting feature is the very shallow slope of the neutron line in relation to the x-ray line. Thus, the RBE is very high at low doses and relatively low at the higher doses (Table I).

For an effect that is produced by a single event (e.g., the passage of an individual directly ionizing particle), a logarithmic plot of effect frequency vs. dose must be a straight line of slope 1 at low doses [7]. In Figs. 4 and 5, the x-ray line has a slope steeper than 1, indicating that in the dose range used, more than one secondary electron was required to induce the effect [7]. The situation for the blue giant cell type is seemingly more complex since the neutron line clearly has a slope less than 1. Unfortunately, we have little knowledge of the nature of the blue giant cells, except that they contain more than the normal amount of DNA [23].

As shown in Figs. 4 and 5 and Table I, RBE values change over a wide range of doses and are found to decrease with increasing doses of radiation. Because of this, no single RBE value should be given for a particular type of aberration unless it is certain that further increases in RBE will not occur if dose is decreased further.

B. Influence of Dose on RBE

RBE was found to change with dose for a variety of experimental end points and in a variety of systems. It has been suggested that this change occurs because elementary lesions causing the radiation effects increase with the square of the x-ray dose but are proportional to neutron dose [24-26].

The dose-response curves (Figs. 4,5) suggest that RBE would become even higher if dose is reduced sufficiently. We investigated this problem using pink mutations as end points (Fig. 6) [8]. The neutron data from about 10 mrads to about 8 rads can be fitted to a slope of +1. The x-ray line displays some curvilinearity but can be approximated by two straight line segments, one with a computed slope (not shown) of +1.4 from about 5 to 100 rads, the other with a slope of +1 from 0.25 to about 5 rads. No evidence of thresholds were seen for either type of radiation. The dashed lines shown in Fig. 6 are theoretical and are explained elsewhere [8]. From about 5 to 100 rads, RBE decreases with increasing x-ray dose from a value of about 50 down to a value of about 15. With higher doses, x rays become more efficient per rad relative to neutrons. Below x-ray doses of about 5 rads, the RBE does not increase indefinitely but remains constant at about 50.

The theory of dual radiation action [25] predicts that RBE should become constant at low doses, and this is clearly shown in Fig. 6. A similar effect occurred in the x-ray curve for colorless mutations as well (Fig. 7). The data, however, are not as good as for pink mutations and data were not collected at extremely low x-ray doses. Since our observations in Tradescantia clone 02 were made, similar changes in slope of the x-ray curves at low doses have been noted in other clones of Tradescantia for both the pink and colorless mutations, and by Smith *et al.* [27] following irradiation of dry maize seeds and scoring yellow-green sectors in leaves 4 and 5. In maize, RBE reached a maximum of about 180 and the maize curve became linear between 2000-3000 rads of x rays. Apparently, the departure from linearity depends on the inherent sensitivity of the system. In clone 02, the dose-response curve departs from linearity at about 5 rads when the frequency of radiation-induced mutations is only about five times the background level or spontaneous frequency [8].

While the change in slope of the x-ray curve allows the definition of a maximum RBE, minimum RBE for clone 02 is of necessity defined as the ratio of doses before the mutation frequency reaches a saturation level. RBE for a specific mutant type cannot be decreased further than the value reached at the saturation point on the upper part of the dose-response curves.

C. Effect of Dose Rate on RBE

In some neutron experiments, it was necessary to vary the dose rate. Although a dose-rate effect was not expected, cuttings were irradiated with 0.55 rads of 0.43-MeV neutrons at dose rates of 0.22 and 60.1 rads/hr (a factor of over 270) as a check. No dose-rate effect was evident. However, the situation is quite different for x rays in which dose rate has a definite influence on RBE.

Figure 8 is identical to Fig. 6 except the linear portion of the x-ray curve has been extrapolated upward and the data are fitted to a computed slope. The lower, linear portion of the curve would be expected to be independent of dose rate, or conversely, dose-rate effects would be most easily found in the range of higher doses on the steeper part of the curve where the quadratic component predominates. It was thought that if dose rate could be reduced sufficiently, the entire x-ray curve might become linear. If this occurs, then the neutron and x-ray curves both would be parallel, hence RBE would not change with dose but would be constant and maximum along their entire length. The greatest dose-rate effect would be expected to occur (or be most easily detected) in the higher dose regions of the curve, about 70-100 rads.

Figure 9 shows the frequency of pink mutant events per hair per rad plotted against dose rate in rads/min. The point of interest here is that at higher dose rates, from about 30 rads/min up to 500 rads/min, relatively little change in mutant event frequency occurs. The mutation frequency either levels off or declines after a dose rate of about 100 rads/min. Thus, within the experimental errors involved, dose rates of 30-500 rads/min would have little effect on the shape of the dose-response curve [28]. Since most of our x-ray experiments were done at 30 rads/min, it is possible that higher yields of mutations and a slightly steeper slope would have been obtained if they had been done at 100 rads/min, but the difference would probably be slight. Along the rest of the curve (Fig. 9) there is a pronounced dose-rate effect [28].

Pink mutant event frequency per hair plotted against x-ray dose for dose rates of 30, 5, and 0.5 rads/min is shown in Fig. 10. The 30 rad/min line includes data from Figs. 6 and 8 and the dashed line is the extrapolated part of the linear part of the x-ray dose-response curve shown in double logarithmic presentation in Fig. 8. Figure 10 is shown in a linear rather than a logarithmic presentation to emphasize dose regions where the influence of dose rate is more prominently detected. It is evident that as dose is decreased, dose-rate effects become less pronounced and at very low doses (about 5-10 rads) would be less detectable or non-existent and appear linear on the logarithmic presentation in Fig. 8. It is quite evident that we can, in fact, approach a linear relationship by reducing dose rate further. However, as dose rate is reduced to these low levels, doses of only about 25 rads or so can be given before the average mitotic cycle time (approximately 24 hr) of *Tradescantia* is exceeded.

Dose rate, as it affects RBE, is summarized in Fig. 11, which shows diagrammatically a linear neutron line and our standard x-ray curve. As dose rate is reduced, the quadratic component, prominent at high doses of x rays becomes reduced so that the entire x-ray dose-response curve tends to become linear. As dose rate is reduced, RBE becomes less dose-dependent and presumably will become maximal and constant throughout the entire range of acute doses that can be given for this particular end point before one mitotic cycle time is exceeded.

D. The Influence of Neutron Energy on RBE

Evidence is accumulating that the energy of fast neutrons is an important factor determining RBE [1-4,18,22,26,29-42]. To investigate this in *Tradescantia*, neutron exposures were made at 12 energy levels ranging from 0.065 to 13.4-MeV [43]. The dose series was chosen so that the lowest doses would be in the region where the x-ray dose-response curve becomes linear (about 5 rads). However, we made no attempt to determine the region of saturation (minimum RBE).

Dose-response curves for pink mutant events per hair (minus control) for each neutron energy are shown as double logarithmic plots in Figs. 12 and 13 relative to 250-kVp x-ray data. The slope of the dose-response curves for each of the energies is linear, that is, the computed slopes did not differ significantly from slopes of 1. RBE values were determined at three different positions along the dose-response curves at mutant event frequencies of 0.003, 0.01 and 0.03 events per hair (Table II). The maximum RBE, opposite 5 rads on the x-ray curve, occurs at a pink mutant event frequency (minus control) of 0.003 events per hair (or below). For all neutron energies, RBE decreases with increasing dose (above about 5 rads of x rays) and increasing mutant event frequency.

Maximum RBE values taken at 0.003 mutant events per hair (Table II) are shown plotted against neutron energy in Fig. 14. RBE as a function of neutron energy increases rapidly from 14.6 at 0.065-MeV up to a peak of 47.6 at 0.43-MeV neutrons. RBE then decreases rapidly to 18.5 at 1.02-MeV neutrons and then to 10.4 at 13.4-MeV. In the region of slow decline, neutron energy increased by a factor of about 13 whereas RBE decreased only by a factor of about 1.8.

Such changes in RBE with neutron energy have been reported for a variety of systems. For example, in mammalian systems, Bateman et al. [1,32-35] report a decrease in RBE in mice with increasing neutron energy for thymus-weight reduction, spleen-weight reduction, spermatogonia depletion and mouse lens opacification. They did not, however, use energies lower than 0.43-MeV. Barendsen [30], Barendsen and Broerse [31], and Broerse et al. [3] found that the largest RBE for loss of reproductive capacity of mammalian cells occurs at 1-MeV in a series of energies from about 1 to 15-MeV. In plant systems, Conger et al. [36] and Smith and Rossi [42] found that RBE decreased as energy increased.

The study most closely related to this present work was reported by Dennis [38] and Dennis and Boot [39], who studied pink mutations in stamen hairs of Tradescantia sp. clone 02 using a series of seven neutron energies from 0.12 to 14.7-MeV and 200-keV x rays. Considering that there are experimental differences involved, their data can be considered in most cases in reasonable agreement with ours.

Hall et al. [4,40] studied inhibition of root growth of seedlings of Vicia faba employing a series of neutron energies ranging from a 60-keV spectrum to 15.4-MeV. Their resulting curve is similar in many respects to Fig. 14 except that there was no pronounced peak at 0.43-MeV. The highest RBE for Vicia faba occurred at 0.35-MeV. We are uncertain why such a pronounced peak occurs in our material and are attempting to clarify this situation. The data of Hall et al. [40] were found to be in substantial agreement with the dependence of RBE on neutron energy predicted from microdosimetric data by Kellerer and Rossi [44]. Our data have not yet been analyzed as rigorously.

E. The RBE of 3.9-GeV Nitrogen Ions

RBE values for a 3.9-GeV nitrogen ion beam generated at the Princeton Particle Accelerator were determined at various positions along the depth-dose curve and at graduated increments in regions close to the Bragg peak [45]. These experiments necessitated certain accommodations, for example, unrooted cuttings were used and these were held in the beam by a specially designed apparatus. Dosimetry was performed by Dr. W. Gross and associates [46]. Several days after irradiation, the flower buds and petals were examined for any unusual phenomena which might be attributed to any unique effects of heavy particle bombardment, but the differences in response between these experiments and those done with neutrons and x rays were only quantitative. Typical results obtained from four positions along the nitrogen depth-dose curve are compared with a 0.43-MeV neutron curve and x-ray curve in Fig. 15. The slopes of the dose-response curves for pink mutant events in stamen hairs do not deviate significantly from 1. In all cases, the lines indicate that RBE increases with decreasing dose and becomes maximal at doses around 5 rads of x rays.

Curve no. 1 (Fig. 15) on the pre-Bragg plateau (upstream in the beam) has a maximum RBE of 3.8. The exposure behind the Bragg peak (no. 4, Fig. 15) indicates a maximum RBE of 5.6. The quality of the radiation in this region is poorly defined, but since the data indicate a linear dependence on dose, the effective radiation probably consists primarily of high LET components.

The data also indicate that the maximum RBE *at* the Bragg peak (14.3) is lower than the maximum RBE of 16.7 *near* the Bragg peak. Presumably, this effect could be due to excess ionization density which might occur in specimens located directly at the Bragg peak. This observation prompted the irradiation of additional specimens to determine the location of maximum RBE in regions close to the Bragg peak. Cuttings were irradiated at the Bragg peak and at three additional increments in front of the peak by successive removal of 2-mm Lucite absorbers. The results suggested that the highest RBE occurred 2 mm upstream from the peak. These RBE values together with their position in relation to the depth-dose curve for nitrogen are shown in Fig. 16. As with neutron experiments, these *Tradescantia* RBE values are higher than those usually reported using mammalian cells. For example, Hall and Lehnert [47] using the same beam found the RBE for 50% cell survival in Chinese hamster cells to be approximately 6 near the Bragg peak.

These nitrogen ion experiments supplied useful data with doses as low as 0.31 rads with ease. This shows that *Tradescantia* experiments may be performed with heavy ion beams at very low doses or dose rates which might make them uniquely useful for detection of high Z particles and RBE determinations during space flight.

F. The Influence of Hypoxia (Oxygen Enhancement Ratios)

Of particular interest was a report by Dennis and Boot [39] indicating a pronounced dependence of OER on neutron energy using pink mutations in *Tradescantia* stamen hairs. They reported a high OER value at 0.42-MeV which appeared inconsistent with the rest of their data (Fig. 19) and it was suggested that this high OER value might be due to a resonance effect.

For our OER experiments, Mr. L. J. Goodman of RARAF designed a light- and air-tight aluminum fixture, permitting an even distribution of gas flow among the inflorescences, capable of withstanding a vacuum and suitable for use both with monoenergetic neutrons and x rays. When the fixture is used with x rays (250-kVcp), dosimetry is accomplished by the insertion of a small Landsverk chamber among the inflorescences. X-ray exposures were run concurrently with neutron exposures.

The fixture containing the cuttings is subjected to a partial vacuum, brought back to atmospheric pressure and flushed with either breathing air or nitrogen. The vacuum removes gas which otherwise might remain trapped in the bracts of the inflorescences. The procedure is repeated three times and then either air or nitrogen is allowed to flow through the fixture for a period of time, usually about 1 hr, before irradiation.

As shown in Fig. 17, the oxygen concentration (in ppm) within the fixture drops quite rapidly. The inflorescences were subjected to nitrogen for periods of time up to 4 hr and then given 50 rads of x rays. The pink mutant event frequency per hair reaches its lowest level after about 15 min in a nitrogen atmosphere and remains constant for at least 4 hr. Oxygen concentration in the fixture drops below 20 ppm in about 1 hr. Apparently the cuttings are rendered hypoxic easily.

OER values have been determined so far for x rays and neutrons of 0.43-, 0.68-, 1.02- and 5.8-MeV, yielding OER values of 3.2, 1.4, 1.3, 1.5 and 1.6. None of the slopes of the neutron lines differed significantly from 1. Likewise, the slopes of the x-ray curves do not differ from the slope obtained in previous open-air experiments. The x-ray curves and an example of the aerated and hypoxic neutron curves are shown in Fig. 18.

Our OER values obtained so far are shown with other data from experiments in which OER was investigated as a function of neutron energy (Fig. 19). Some 1966 data for human cells [48] do not show much of an OER dependence on neutron energy. However, the data obtained using Vicia faba [40] do show an OER dependence on neutron energy. The greatest dependence, however, occurs for Tradescantia as previously reported by Dennis and Boot [39]. Our Tradescantia data seem consistent with values reported for Vicia faba (also obtained at RARAF) at low energies, but our value of 5.8-MeV is lower than published values for Vicia faba or Tradescantia. However, the most striking difference between the two Tradescantia experiments occurs with OER values at 0.42-0.43-MeV where our OER value (1.4) is in clear disagreement with the value (2.1) reported by Dennis and Boot [39].

One of our main efforts, at present, is the determination of the OER for x rays at very low doses. Obtaining the needed data is proving quite a formidable task, not only because of the large numbers of cuttings required and the time consuming experimental procedures but because the experimental procedure in use tends to increase the spontaneous mutation rate by a factor of about 2. Thus, even larger numbers of stamen hairs must be scored than would be necessary in an open-air system (Fig. 2). This difficulty is especially pronounced in the dose regions at and below 5 rads, where the open-air x-ray dose-response curve becomes linear [8].

The results from these low-dose experiments shown in Fig. 18 suggest that the hypoxic as well as the aerated curve will change from a steep slope and become linear at about 5 rads. It thus appears improbable that OER will change at low doses.

G. The Influence of ICV and Ploidy Level on RBE: Pollen Abortion Data

Itwould be desirable to extend stamen hair studies to several other taxa of Commelinaceae in order to determine the influence of interphase chromosome volume (ICV), nuclear volume (NV) and ploidy level on survival, somatic mutation induction and RBE. However, many of the species having nuclear characteristics suitable for this study either lack stamen hairs or the stocks available at present are all homozygous for flower color. Pollen abortion which has long been recognized as a satisfactory end point for radiobiological studies of this nature [49] provides a suitable alternative [16,50].

After irradiating with x or gamma rays, the percentage of pollen abortion induced was obtained for 15 taxa of four genera of Commelinaceae. Of this number four species of two genera were irradiated with both 0.43-MeV neutrons and x rays so that RBEs could be determined. These species, together with their ICVs and RBE values are given in Table III. Details of this test system are given elsewhere [21]. In general, the handling of the material for irradiations was similar to the general handling of clone 02 cuttings. Clone 02 was not used in these studies because it has an undesirably high spontaneous rate of pollen abortion [50].

After irradiation, fresh pollen is collected daily and spread on a slide in a drop of cotton blue stain [51] and covered with a cover slip. The cotton blue distinguishes nonaborted pollen (blue staining) from aborted pollen (unstained).

From the basic data of percentage abortion vs. time after irradiation for each experiment, it is possible to determine maximal or peak values for pollen abortion. Examples of daily postirradiation pollen abortion responses are shown in Fig. 20.

Since the purpose of these experiments was to make comparisons among species, nuclear volumes of vegetative shoot meristems were measured to determine ICV and a constant ratio between somatic and meiotic nuclear volumes was assumed. It has already been shown that nuclear or chromosomal volumes of shoot meristematic nuclei of different species are directly related to the radiosensitivity of species when the end point is injury to the germinal tissue [16].

Examples of dose-response curves (maximum percentage of pollen abortion vs. dose) are shown in Figs. 21 and 22. From these and similar curves, RBE values were determined at various percentages of pollen abortion along the curve (Table III).

The slopes of the dose-response curves for both the high and low LET radiations can differ for each species. Slopes for low LET radiation were found to be greater than, equal to, or less than 1. Slopes of neutron dose-response curves for all species were less than 1. These variations in slope between radiations and among species do not allow an interpretation that would suggest a general mechanism for interaction between radiation events and the aborted pollen grains so interpretations of the slopes are pending.

Dose-response curves for two highly radiosensitive diploid forms of Tradescantia paludosa and the tetraploid T. virginiana (all with large chromosomes) are shown in Fig. 21. The tetraploid T. paludosa was given only two doses of neutrons, but these data points also can be superimposed on the diploid neutron line (Fig. 21). The additional set of chromosomes does not appear to affect the shape of the dose-response curves significantly nor does the genetic redundancy appear to offer a radioprotective effect [9,11,14].

The slopes of the dose-response curves for the neutron-treated diploid and tetraploid Gibasis are also both less than 1 (Fig. 22). The slopes, however, suggest that if differences in sensitivity that appear to exist at the lower doses are real, then the tetraploid is slightly more sensitive than the diploid. The tetraploid x-irradiated Gibasis shows no consistent differences in radiosensitivity compared to the diploid, so data from both were fitted to a common slope (Fig. 22). The common slope is steeper than 1. The ICVs of both species are fairly similar as would be expected (Tables III, IV). Again the additional set of chromosomes in the tetraploid do not appear to offer any apparent radioprotective effect.

For these species, RBE decreases with increasing dose, although the change is small for Tradescantia subacaulis (Table III). Within the dose range investigated, the highest RBE values occur at 10% pollen abortion and range from 23.1 for the tetraploid Gibasis to 9.4 for the diploid Gibasis. The differences in RBE values among the species studied may be influenced by differences in their ICVs. It has been shown by others that RBE may tend to increase with increasing chromosome volume in some cases [12,52] but not all [53]. For the species studied here, there is a definite trend at 50% pollen abortion between increasing RBE and increasing ICV (Fig. 23). More species should be studied to determine whether a predictable relationship exists between ICV and RBE.

It has been found in higher plants that there is an inverse correlation (-1 slope) between ICV and radiosensitivity [9-16]. Many of these correlations were found using the doses for 50% effect with various end points.

The relationships between ICV, NV and 50% pollen abortion for neutrons and x or gamma rays are shown in Fig. 24. Pertinent data are given in Table IV. There does not appear to be an inverse relationship (-1 slope) between pollen abortion and nuclear volume for either the low LET radiations or neutrons and the correlation coefficients are poor.

However, an inverse relation (-1 slope) exists between 50% pollen abortion and ICV for acute exposure to low LET radiation. Thus, by determining the nuclear volume and chromosome number of meristematic tissue, it is possible to estimate the relative radiosensitivity of the germinal tissue to low LET radiation.

The current data suggest that an inverse relationship between pollen abortion and ICV also holds following neutron irradiation (Fig. 24), but the data are presently too limited to be conclusive with respect to slope.

H. Concluding Remarks

Much of this report involves studies that are still incomplete. Only some of the data have been subjected to rigorous biophysical interpretation. Of importance is the fact that most of the data which involve factors influencing RBE were obtained within one experimental eukaryotic system (or for pollen abortion in closely related species) with substantially less effort than might be required using other systems. The major question is how useful such basic data from plants may be in constructing models and whether basic principles derived from plants can be extrapolated to other systems. The present data suggest that the behavior of _Tradescantia_ in many fundamental respects is essentially the same as that of many other eukaryotic organisms. If this proves to be true, then perhaps more advantage should be taken of its unique characteristics that make it especially useful for studying mutation induction at very low doses or dose rates.

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Table I

RBE Values for Aberrant Events in *Tradescantia* Stamen Hairs Following 0.43-MeV Neutron and 250-KVp X-ray Treatments

Cell type	Aberrant events per hair* (minus control)	Dose (rads) 250-kVp x ray	Dose (rads) 0.43-MeV neutron	RBE
Pink (normal size)	0.1	12.5	0.4	31.3
	0.2	22.5	0.9	25.0
	0.5	50.0	2.7	18.5
	1.0	90.0	6.1	14.8
	1.5	128.0	10.0	12.8
Pink giant	.01	44.0	1.3	33.8
	.02	70.0	2.7	25.9
	.05	128.0	7.2	17.8
	.08	17.0	12.0	14.6
Pink dwarf	.04	46.0	1.7	27.1
	.05	63.0	2.1	30.0
	0.1	90.0	4.3	20.9
	0.2	130.0	8.5	15.3
Colorless (normal size)	.05	12.5	0.27	46.3
	0.1	20.5	0.56	36.6
	0.2	33.0	1.2	27.5
	0.5	61.0	3.2	19.1
	1.0	98.0	6.6	14.8
	1.5	130.0	10.5	12.4
Colorless giant	.01	31.0	0.92	33.7
	.02	51.0	1.75	29.1
	.05	103.0	4.2	24.5
	0.1	175.0	8.0	21.9
	0.15	235.0	11.5	20.4
Colorless dwarf	.02	16.0	0.58	27.6
	.05	26.0	1.15	22.6
	0.1	38.0	1.9	20.0
	0.2	55.0	3.2	17.2
	0.5	90.0	6.3	14.3
	1.0	135.0	10.8	12.5
	1.5	168.0	14.5	11.6
	2.5	220.0	21.0	10.5
Blue giant	.07	31.5	0.19	165.8
	0.1	40.0	0.36	111.1
	0.2	64.0	1.2	53.3
	0.5	120.0	5.8	20.7
	0.8	165.0	13.0	12.7
	1.5	250.0	-	-
	3.0	400.0	-	-
Blue dwarf	0.1	16.0	0.24	66.7
	0.3	43.0	1.35	31.9
	0.5	68.0	3.0	22.7
	0.8	105.0	6.2	16.9
	1.0	128.0	8.8	14.5

*Data are cumulative for days 7 through 16 postirradiation.

(From [22]).

Table II

RBE Values for Pink Mutant Events in *Tradescantia* Stamen Hairs Following Irradiation With Neutrons of Various Energies and 250-kVp X Rays

Neutron energy (MeV)	Pink events per hair* (minus control)	Dose (rads)		
		X ray	Neutron	RBE
0.065	0.003	5.0	0.34	14.7
	0.01	12.0	1.10	10.9
	0.03	27.0	3.30	8.2
0.110	0.003	5.0	0.23	21.7
	0.01	12.0	0.76	15.8
	0.03	27.0	2.30	11.7
0.220	0.003	5.0	0.19	26.3
	0.01	12.0	0.64	18.8
	0.03	27.0	1.90	14.2
0.340	0.003	5.0	0.15	33.3
	0.01	12.0	0.54	22.2
	0.03	27.0	1.60	16.9
0.395	0.003	5.0	0.115	43.5
	0.01	12.0	0.38	31.6
	0.03	27.0	1.15	23.5
0.430	0.003	5.0	0.105	47.6
	0.01	12.0	0.37	32.4
	0.03	27.0	1.10	24.6
0.552	0.003	5.0	0.15	33.3
	0.01	12.0	0.49	24.5
	0.03	27.0	1.45	18.6
0.680	0.003	5.0	0.16	31.3
	0.01	12.0	0.54	22.2
	0.03	27.0	1.65	16.4
1.02	0.003	5.0	0.27	18.5
	0.01	12.0	0.90	13.3
	0.03	27.0	2.70	10.0
2.05	0.003	5.0	0.33	15.2
	0.01	12.0	1.10	10.9
	0.03	27.0	3.40	7.9
6.0	0.003	5.0	0.37	13.5
	0.01	12.0	1.20	10.0
	0.03	27.0	3.70	7.3
13.4	0.003	5.0	0.48	10.4
	0.01	12.0	1.60	7.5
	0.03	27.0	4.80	5.6

Table III

RBE Values Determined for Two Species of *Tradescantia* and *Gibasis* With Pollen Abortion as an End Point

% Pollen abortion	Neutron dose (rads)	X-ray dose (rads)	RBE
Gibasis geniculata (2×) ICV=41.9			
10	3.2	30	9.4
20	7.8	51	6.5
50	24.5	105	4.3
Gibasis karwinskyana (4x) ICV=45.9			
10	1.3	30	23.1
20	4.0	51	12.8
50	17.0	105	6.2
Tradescantia subacaulis (2x) ICV=55.5			
10	0.67	9.5	14.3
20	2.0	25.5	12.8
50	8.8	88.0	10.0
Tradescantia paludosa (No. 2465) (2x) ICV=75.5			
10	0.67	13	19.4
20	1.8	28	15.6
50	6.5	76	11.7

(From [21]).

Table IV

Nuclear Characteristics and Doses to Produce 50% Pollen Abortion Determined From Dose-Response Curves

Species	Species codes	Chromosome number (2n)	Ploidy level (x)	Nuclear volume (μ^3)	ICV (μ^3)	50% Pollen abortion	
						X ray dose (rads)	Neutron dose (rads)
Floscopa scandens	1	54	6	538.7	10.0	880	–
Gibasis geniculata	2	16	2	670.5	41.9	105	24.5
G. karwinskyana (1794)	3	20	4	913.6	45.7	105	17
G. kawinskyana (1803)	4	20	4	786.2	39.3	–	–
Tradescantia sp. (2084)	5	29	C.2	918.0	31.6	265	–
T. blossfeldiana	6	72	12	504.0	7.0	600	–
T. commelinoides	7	16	2	621.0	38.8	225	–
T. crassula	8	72	12	506.0	6.8	490	–
T. paludosa (B2-2)	9	12	2	787.1	65.6	90	–
T. paludosa (2465)	10	12	2	905.8	75.5	76	6.5
T. paludosa (4x)	11	24	4	1330.0	55.4	–	–
T. subacaulis	12	12	2	664.5	55.5	88	8.8
T. virginiana	13	24	4	1381.0	57.5	90	–
Tripogandra elongata	14	64	8	488.9	7.7	700	–
T. glandulosa	15	16	2	305.8	19.1	–	24

(From [21]).

FIGURE LEGENDS

Fig. 1. Plot of per cent hairs with pink colorless events vs. days postirradiation. Stamen hairs are routinely scored for mutations from day 11 through 15 postirradiation when mutation frequency is highest.

Fig. 2. Guide for predicting population size required at various doses of x rays to determine Tradescantia clone 02 pink event frequency (minus control) with a standard error which is a predetermined percentage of the mean. Graph is based on data collected during a 5-day scoring period grown under our standard growth chamber conditions (days 11-15 postirradiation, assuming 300 hairs per flower). (From [17]).

Fig. 3. Survival curves for stamen hairs after neutron and x irradiation. The surviving fraction is the percent of normal length hairs as percent of control for the terminal third of the filament. The surviving fraction consists of hairs both with and without aberrations. (From [17).

Fig. 4. Neutron (0.43-MeV) and x-ray dose-response curves for pink mutant events in stamen hairs. Data are cumulative for day 7 through 16 postirradiation. Open symbols are saturation points.

Fig. 5. Neutron (0.43-MeV) and x-ray dose-response curves for blue giant cells in stamen hairs. Data are cumulative for day 7 through 16 postirradiation.

Fig. 6. Neutron and x-ray dose-response curves for pink mutant events per hair (minus control) in stamen hairs. The points represent average values obtained by dividing the total number of mutant events by the total number of stamen hairs scored from day 11 through 15 postirradiation. The open symbols are saturation points and were not used in computing the slopes. The dashed lines represent theoretical slopes as detailed in Sparrow et al. [8]. The background (control) frequency is indicated. (From [8]).

Fig. 7. Neutron and x-ray dose-response curves for colorless mutant events per hair (minus control) in stamen hairs. The points represent average values obtained by dividing the total number of mutant events by the total number of stamen hairs scored from day 11 through 15 postirradiation. The open symbols are saturation points and were not used in computing slopes.

Fig. 8. Plot of data from Fig. 6 but with best fitting slopes. Linear portion of the x-ray dose-response curve has been extrapolated upward.

Fig. 9. Plot of pink mutant events per hair per rad vs. dose rate (derived from [28]).

Fig. 10. Dose-response curves for pink mutant events per hair following x irradiation at 30, 5.0 and 0.5 rads/min. Top and bottom lines were transposed from Fig. 8. The bottom line represents the extrapolated linear portion of the dose-response curve of Fig. 8 (derived from [28]).

Fig. 11. Diagram illustrating changes in RBE as dose rate is changed for pink mutant events in stamen hairs of clone 02.

Fig. 12. Plots of pink mutant events per hair (minus control) vs. dose for 250-kVp x rays and neutrons ranging in energy from 0.065 to 0.43-MeV. Arrows indicate region on the dose-response curves where RBE is maximum.

Fig. 13. Plots of pink mutant events per hair (minus control) vs. dose for 250-kVp x rays and neutrons ranging in energy from 0.552 to 13.4-MeV. Arrows indicate region on the dose-response curves where RBE is maximum.

Fig. 14. Plot of maximum RBE for pink mutant events in stamen hairs vs. neutron energy.

Fig. 15. Plot of pink mutant events per hair vs. nitrogen ion dose. Responses at all four positions are linear. Slopes 2 and 4 have been extrapolated (dashed lines) to the region of maximum RBE (opposite 5 rads of x rays). X-ray and neutron lines are included for comparison. (From [45]).

Fig. 16. Depth-dose curve for 3.9-GeV nitrogen ions from the Princeton Particle Accelerator. Inset scale for the Bragg peak has been expanded 10X. Numbers above curve indicate position of the inflorescences in relation to the peak. Numbers below curve are maximum RBE values determined at these positions. (From [45]).

Fig. 17. Plot of pink mutant events per hair (minus control) and ppm oxygen vs. time in minutes of nitrogen flow. X-ray dose was 50 rads.

Fig. 18. Plot of pink mutant events per hair (minus control) for 1.02-MeV neutrons and 250-kVcp x rays irradiated under air and nitrogen atmospheres. The slopes of the neutron lines are +1. The x-ray curves have a steeper slope. The low-dose region of the x-ray curves has not been resolved, but available data suggest OER will not change at low doses.

Fig. 19. Plot of oxygen enhancement ratios (OER) vs. neutron energy for human cells, *Vicia faba* and *Tradescantia*. The OER values for *Tradescantia* at 0.42-0.43-MeV are clearly discrepant.

Fig. 20. Percent pollen abortion (minus control) vs. days postirradiation for *Gibasis geniculata* following x-ray (a) and neutron (b) irradiation. From these and similar data the maximum percent pollen abortion may be determined for each exposure. (From [21]).

Fig. 21. Dose-response curves for maximum percent pollen abortion (minus control) following neutron and low LET radiation for diploid and tetraploid *Tradescantia* spp. (From [21]).

Fig. 22. Dose-response curves for maximum percent pollen abortion (minus control) following neutrons and x irradiation for diploid and tetraploid *Gibasis* spp. (From [21]).

Fig. 23. RBE at 50%pollen abortion vs. ICV for four species. (From [21]).

Fig. 24. Doses required to produce 50% pollen abortion following low LET and neutron irradiation vs. ICV and NV for several species. The natural slopes with ICV as a parameter are not significantly different from -1. See Table IV for species code, doses, ICV and NV. (From [21]).

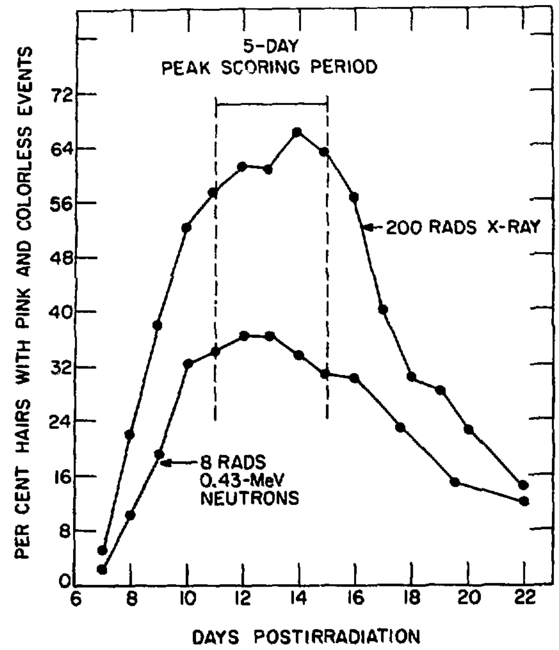


FIGURE 1

SCORING GUIDE FOR PREDICTING POPULATION SIZE REQUIRED TO DETERMINE *Tradescantia* CLONE 02 PINK EVENT FREQUENCY (MINUS CONTROL) WITH A S.E. WHICH IS A PREDETERMINED PERCENTAGE OF THE MEAN

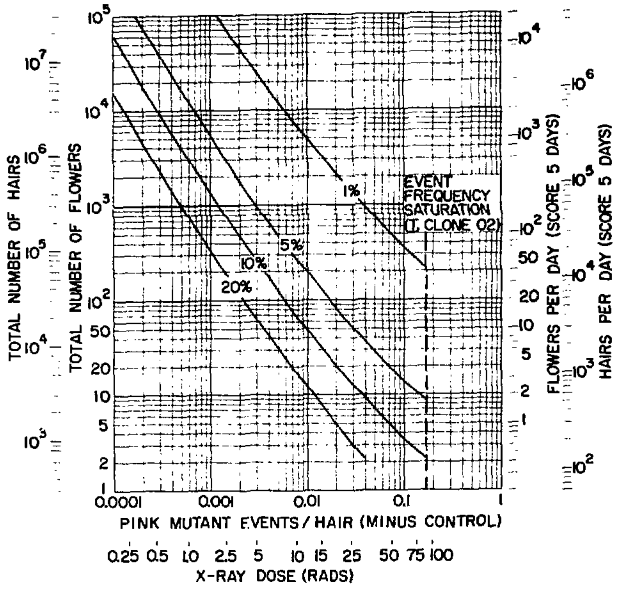


FIGURE 2

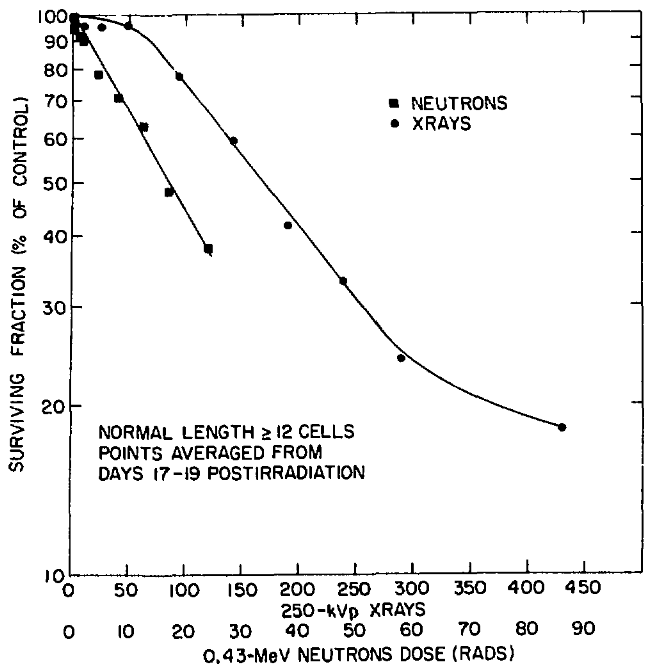
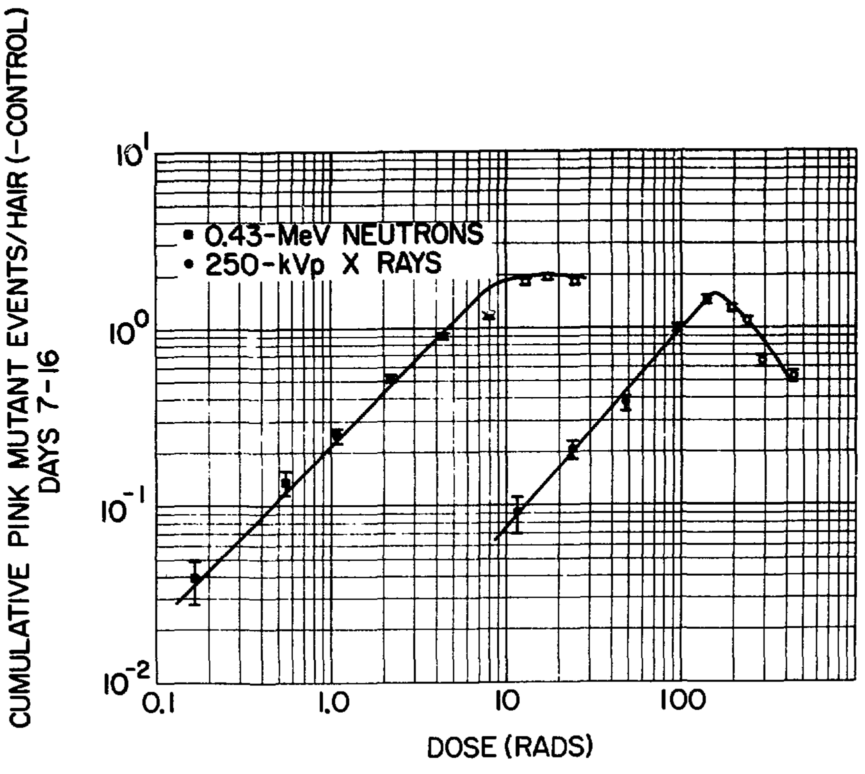


FIGURE 3



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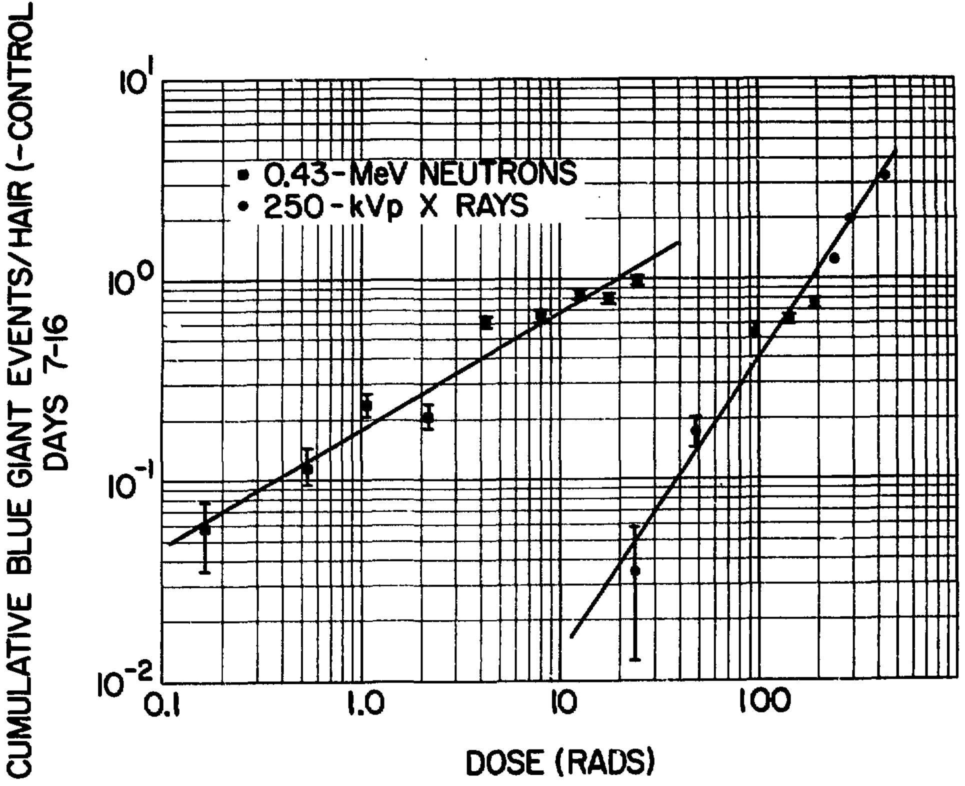


FIGURE 5

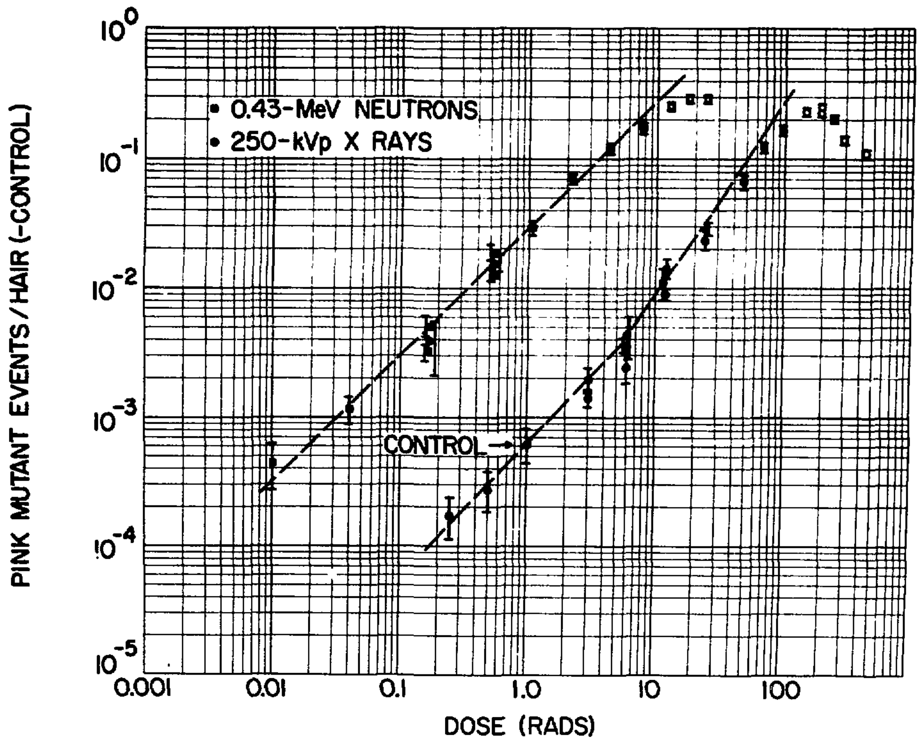


FIGURE 6

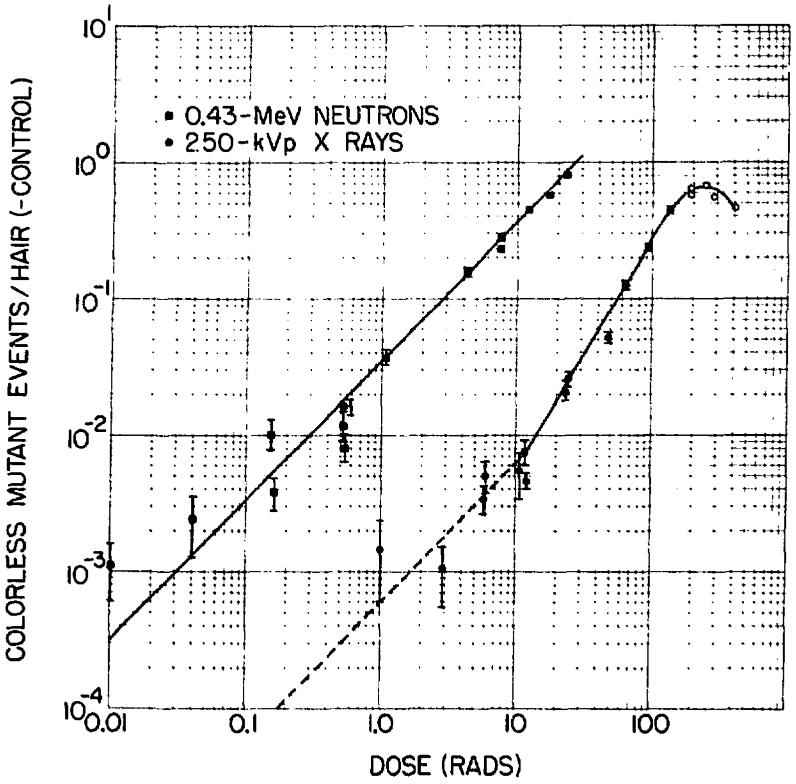


FIGURE 7

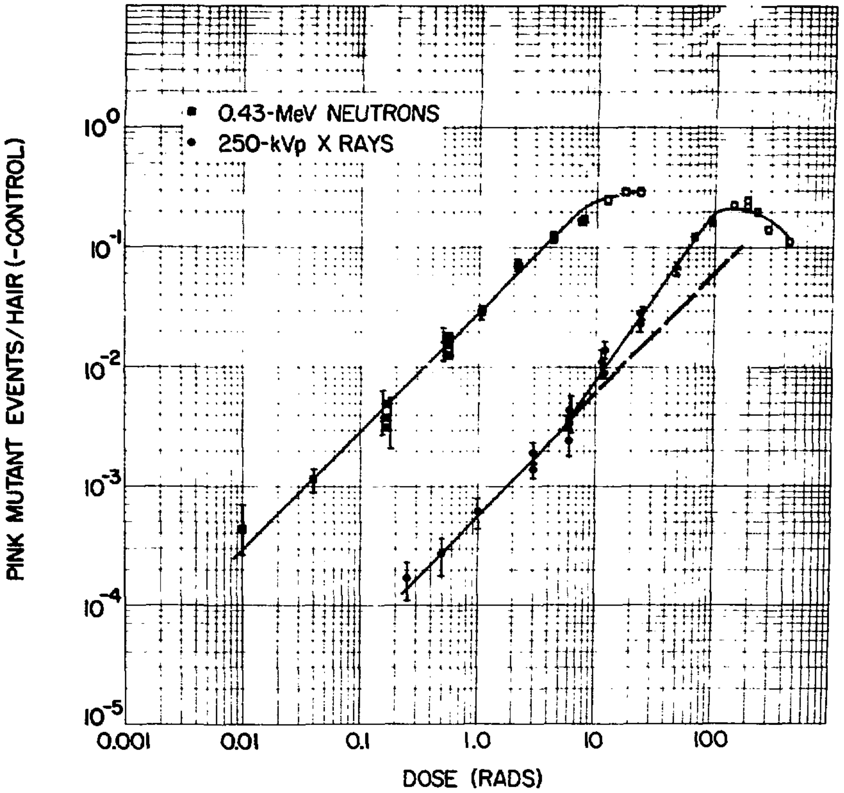


FIGURE 8

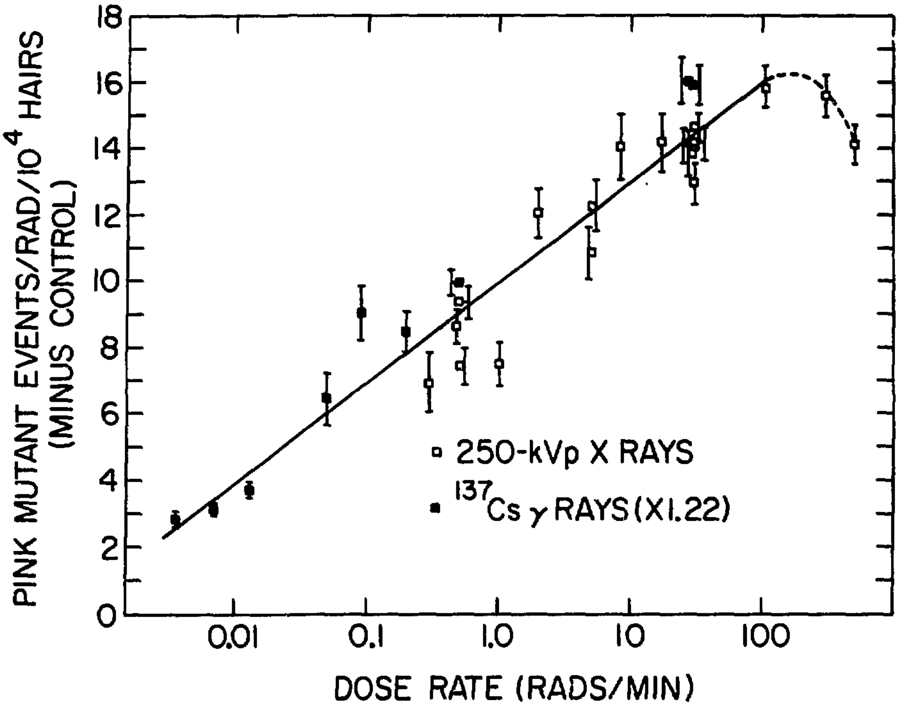


FIGURE 9

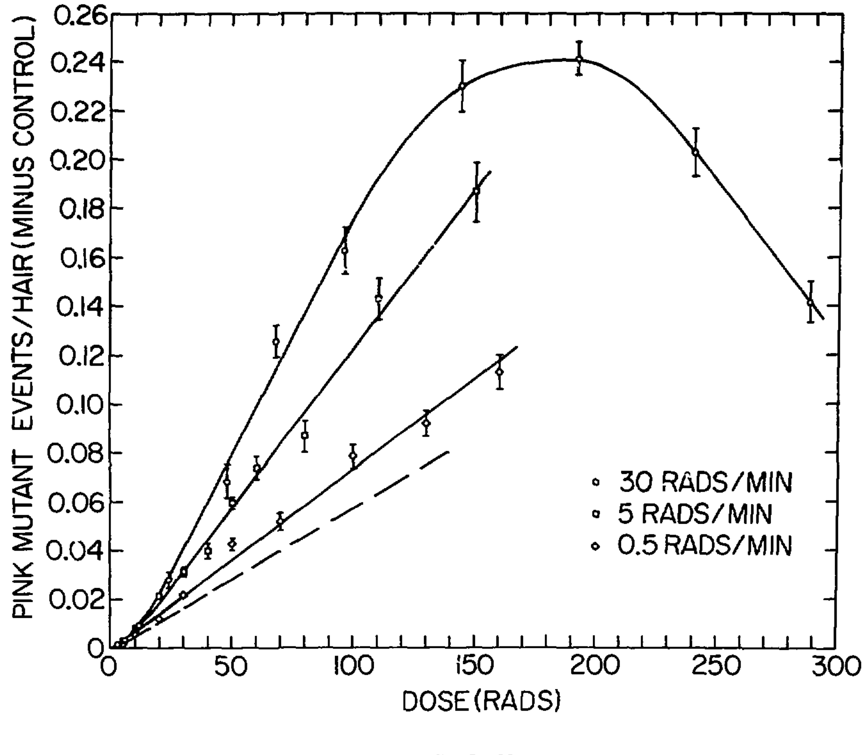


FIGURE 10

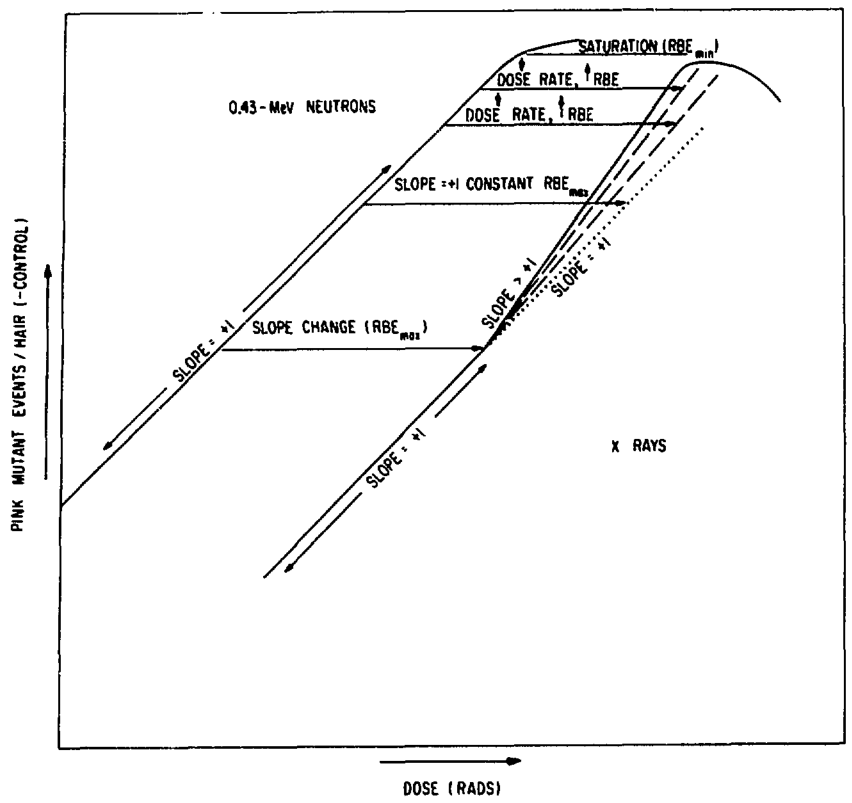


FIGURE 11

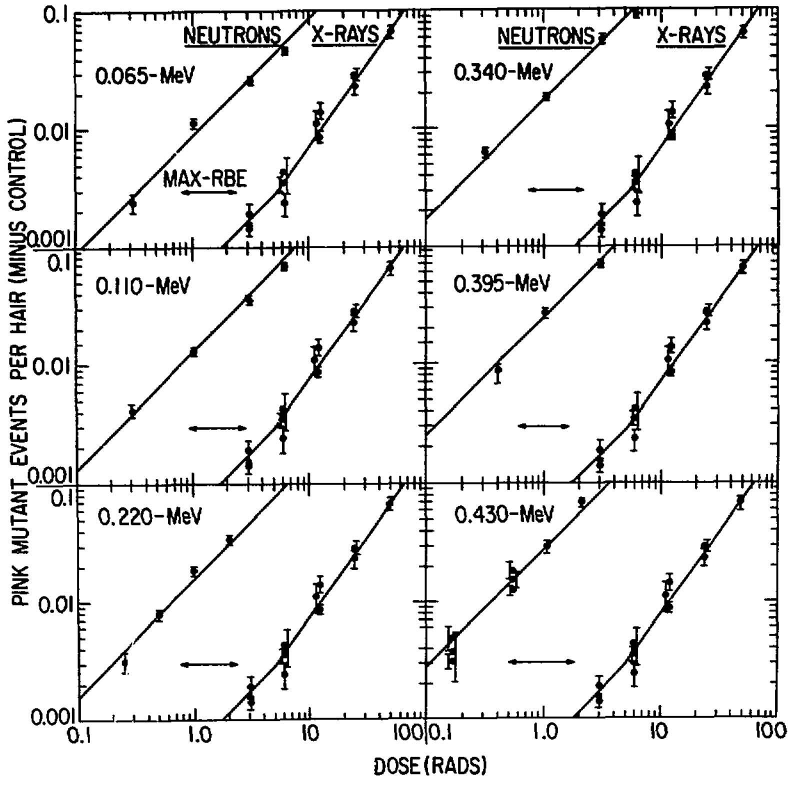


FIGURE 12

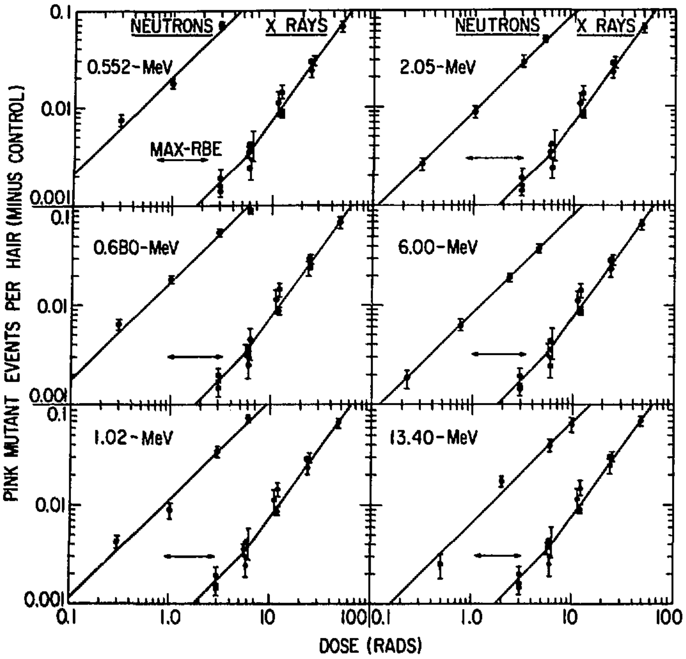
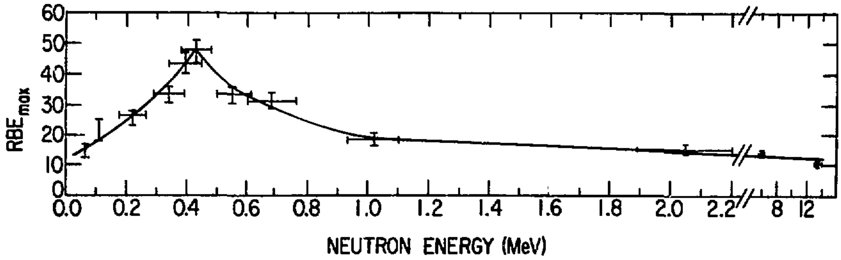


FIGURE 13



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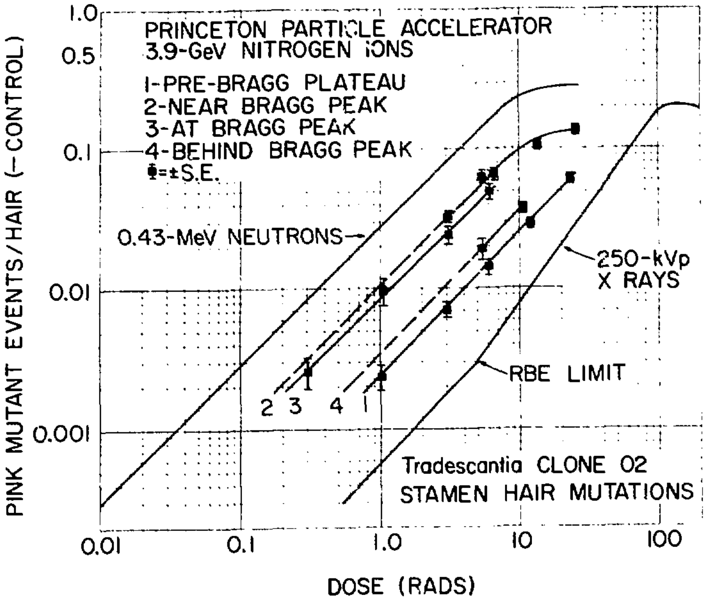

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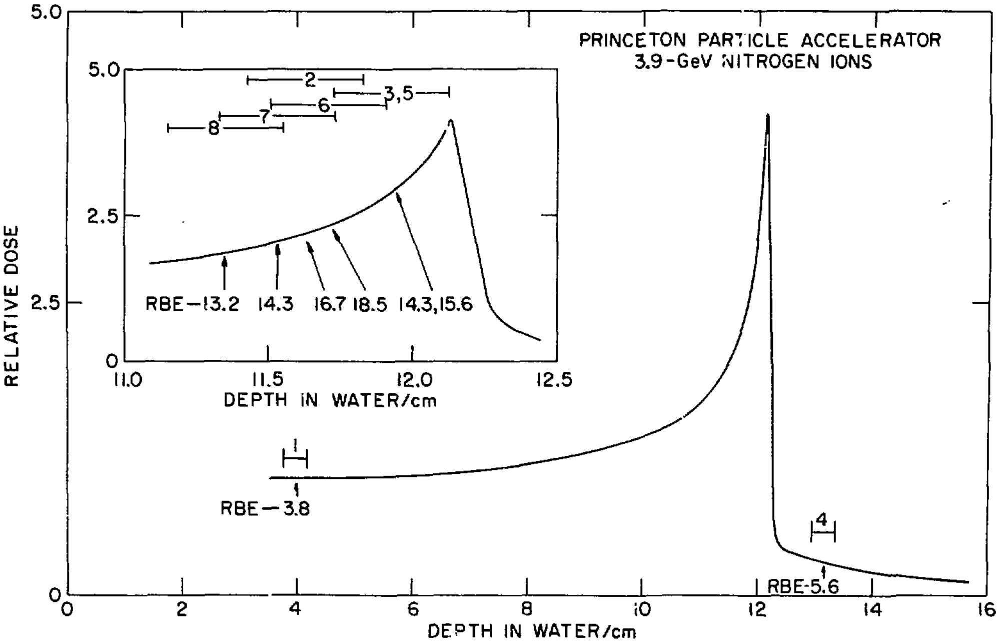

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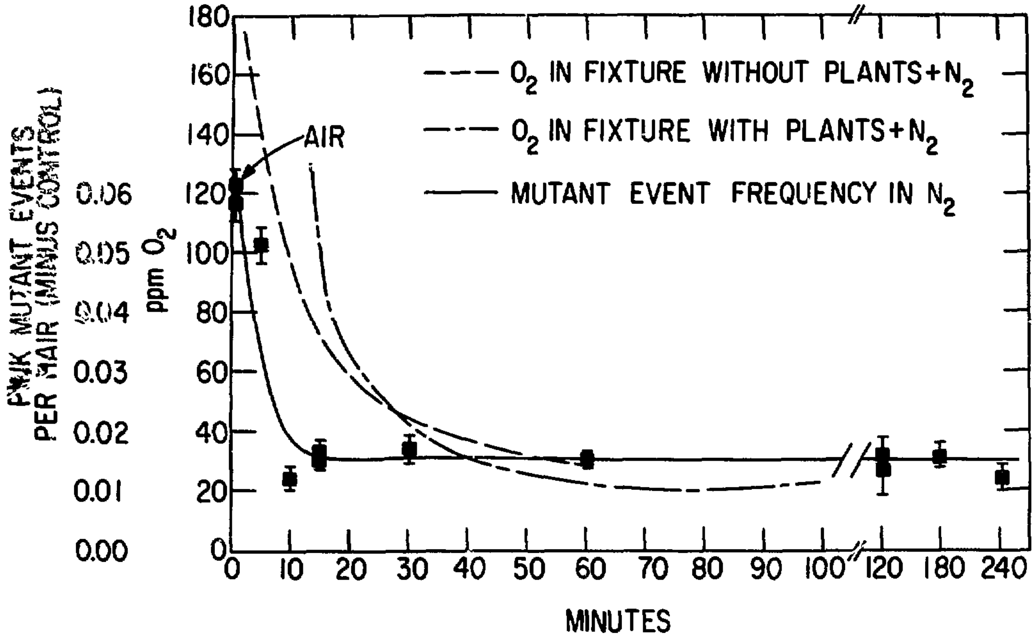

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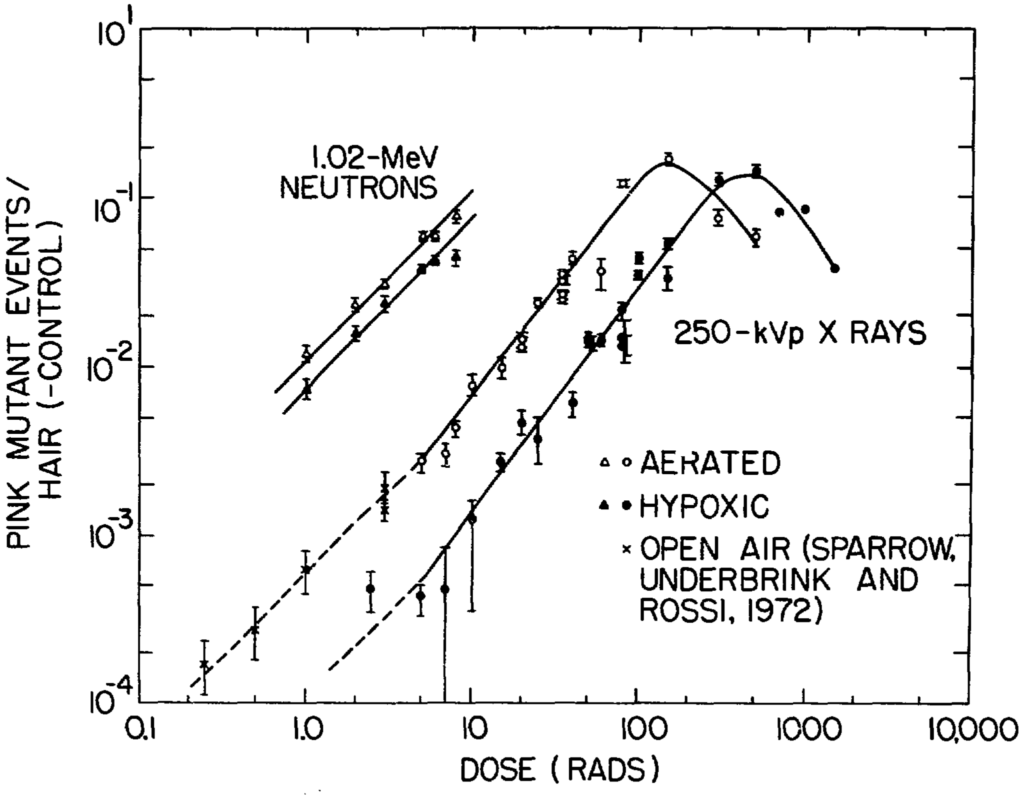

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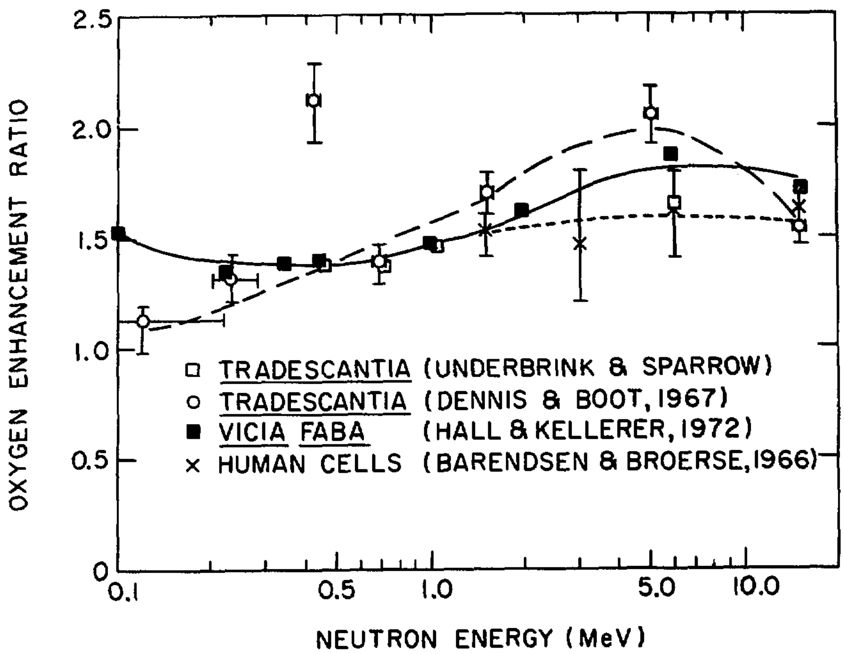

FIGURE 19

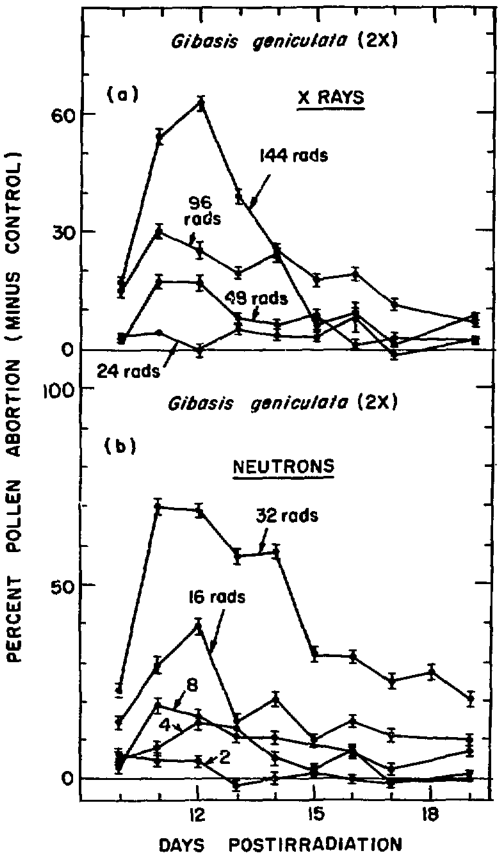

FIGURE 20

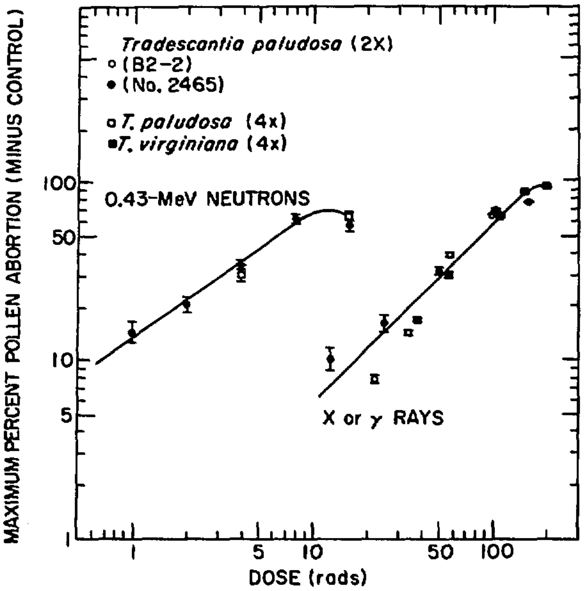

FIGURE 21

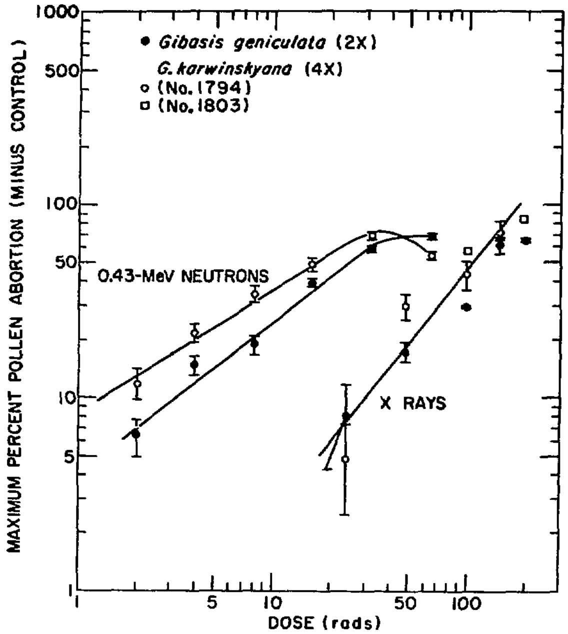

FIGURE 22

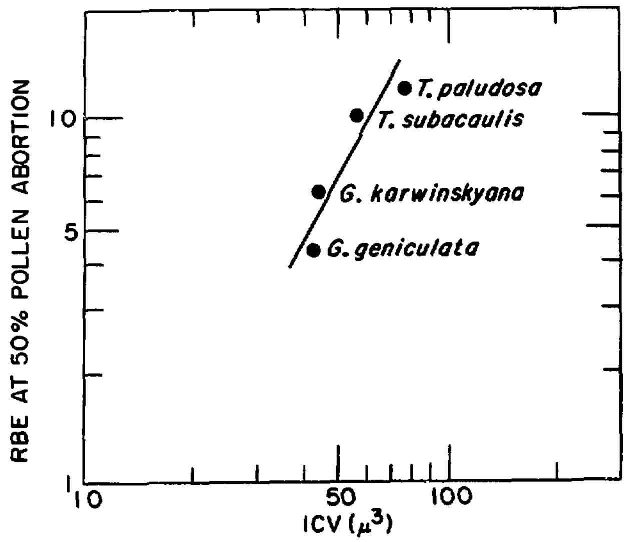

FIGURE 23

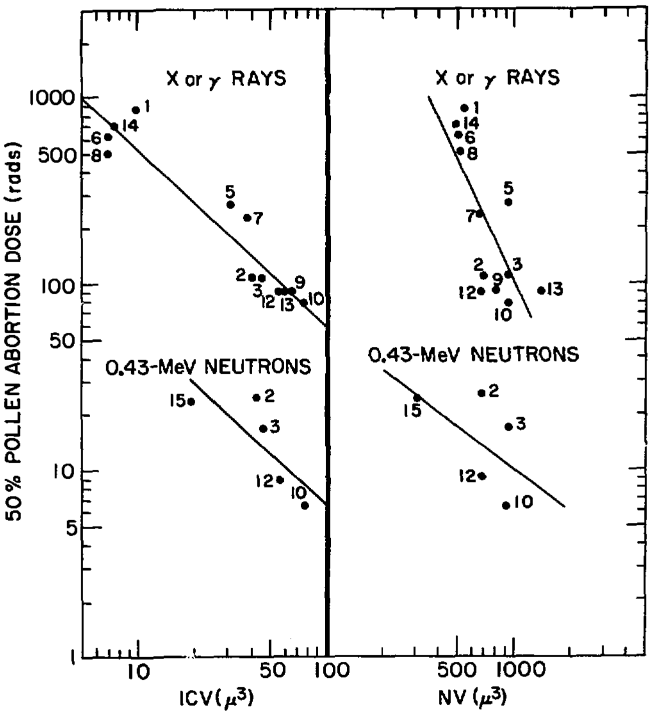

FIGURE 24