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DISCLAIMER

THE RBE OF NEGATIVE PIONS IN 2-DAY-OLD ASCITES TUMORS[1]

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ABSTRACT

A previous report gave the relative biological effectiveness (RBE) of a 90-MeV negative pion beam at the peak of the depth-dose distribution compared with ^{60}Co rays, for a 5-day-old tumor; the value was found to be 5.4±1.8. In the investigation presented here, the RBE of negative pions was measured for a 2-day-old lymphoma.

Partial pressure of oxygen (pO_2) was measured *in vivo* with a microelectrode. The 5-day-old tumors were found to be hypoxic; the 2-day old tumors showed a pO_2 of about 7.0 mm Hg.

The dose rate of pions at the peak position was increased in this experiment to about 25–30 rads/hr by focusing the beam. The resulting beam was small, and only one mouse at a time could be irradiated. Mice were exposed to doses ranging between 60 and 150 rads. After irradiation, mice were sacrificed, cells withdrawn and counted, and serial dilutions prepared from each irradiated mouse and from nonirradiated controls. Each concentration was injected intraperitoneally into a group of 10 female LAF_1 mice; at the end of 8 weeks the TD_{50} (the number of tumor cells needed to produce 50% takes) for each assay was evaluated. The ratios of the TD_{50} for controls to the $TD_{50's}$ for the different doses gave the surviving fractions, from which the survival curve was drawn. A curve with a small shoulder was obtained: D_0 = 76 rads, and D_Q = 44 rads. By comparing with survival obtained by irradiation with ^{60}Co γ rays at

150 rads/hr, the RBE was estimated to be 5.4±0.6; but this value was only 2.9±0.5 when the estimate was made on the basis of ^{60}Co γ-ray irradiations at a dose rate of 45 rads/min.

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KEY WORDS: RBE, negative pions, ascites, lymphoma.

INTRODUCTION

The relative biological effectiveness (RBE) for negative pions (π^-) at the peak of depth-dose distribution on 5-day-old lymphoma ascites tumor cells has been reported. The RBE was found to be 5.4 when proliferative capacity was used as a biological end point (1), and 2.5 when polyploidy induction was the end point (2).

The potential use of π^- in future cancer therapy motivated us to do as many experiments as feasible with the beam available at the 184-inch synchrocyclotron at Berkeley. In the series of experiments reported here attempts were made to obtain more information about the RBE, and to gain some insight into the oxygen enhancement ratio (OER).

MATERIALS AND METHODS

Five-day-old ascites tumor cells induced by an inoculum of 10^6 cells were used in previous experiments. An unfocused pion beam was used and, hence, eight mice could be exposed at a time. The pion exposures were as long as 40 hours, which made the tumors almost 7 days old by the end of the irradiation.

Measurements of the partial pressure of oxygen (pO_2) *in vivo* showed that 5-day old tumors were hypoxic and, in most cases, anoxic. Two- and 3-day-old tumors, even with an inoculum of 10^7 cells, always showed that oxygen was available to the cells.

Two-day-old tumors (inoculum: 10^7 cells) were used in the investigation reported here to measure the RBE and to get some information on the OER for these cells *in vivo*. Experiments were also done with ^{60}Co γ-rays at a dose rate equivalent to the pion dose rate multiplied by 5.0 (round value of previously determined RBE) for comparison. In addition, acute exposures of ^{60}Co γ-rays, as well as 910-MeV helium ions, were also used to study the effects of dose rates on this system.

The π^- beam had a contamination of 25% electrons and 10% muons. The dose rate of the beam was increased by focusing it to give 25–30 rads/hr over a useful cylindrical volume of 2.5 cm diameter and 5.0 cm height. The depth-dose distribution of the beam was measured by using a small tissue-equivalent ionization chamber (0.75 ml) filled with air; this distribution is shown in Fig. 1.

A cylindrical holder was made of 1.5-mm-thick Lucite, with an outside diameter of 2.5 cm. A mouse could be confined within the desired height of 5 cm (or less) by means of a piston. Several holes in the holder provided good ventilation for the mouse. The arrows in Fig. 1 mark the limits of the position of the cylindrical holder during irradiation. One mouse could be irradiated at a time; due to cyclotron time limitations, the mice were exposed at only three dose points. Figure 2 shows the schematic setup for exposures. The cylindrical holder was placed at the peak of the depth-dose distribution; in the position of beam maximum [located by determining the horizontal and vertical beam profiles with a small tissue-equivalent ionization chamber (3)]. For each exposure the variation in dose received by the tumor was estimated to be no more than 20%. The holder was rotated at 10 revolutions per minute during irradiation.

The procedure and end point were the same as previously described (1), namely, the <u>in vivo</u> irradiation of ascites tumors, and the test for tumor-forming ability of the cells when injected into normal mice.

LAF_1 female mice (Jackson Laboratory, Bar Harbor, Maine) were injected intraperitoneally with 10^7 lymphoma cells (our designation, L-2) 2 days prior to irradiation time; they were placed in the holder and irradiated, one at a time, for 3, 4, and 6 hours. Neither food nor water was given during irradiation. Control experiments were carried out similarly, except for the ^{60}Co γ-ray exposures, for which we used different holders in order to have four animals per dose; the mice were confined in a similar enclosure, but they were not rotated.

Control and irradiated animals were sacrificed with ether, cells were withdrawn and counted with the hemocytometer, and fivefold dilutions were prepared for serial injection into LAF_1 female mice, 12 to 16 weeks old, weighing 20±3 g. Ten animals were used for each dilution, and five dilutions used for each assay. The tumor "take" was evaluated at the end of 8 weeks, and the TD_{50} (the number of cells necessary to produce tumors in 50% of the mice) was estimated by Litchfield and Wilcoxon's method (4). This method, and the use of the TD_{50} as an end point, have been discussed most recently by Elkind and Whitmore (5), Hewitt et al. (6), and Kallman (7). The ratios of the TD_{50} for the control group to the TD_{50}'s obtained for each irradiated group gave the surviving fractions, from which the survival curve was drawn. Curve fitting was done by eye, and propagation of errors for the TD_{50} ratios gave asymmetrical 95% confidence intervals for the surviving fractions. Since only one experiment was done, an estimate of the standard error was obtained by taking one-fourth of the 95% confidence interval for each surviving fraction (8, 9). The mean lethal dose, D_0, the dose required to reduce survival from 1 to 0.37 in the exponential region of survival curves, was then estimated from the graphs, as well as the standard error. The RBE was estimated on the basis of 0.1 survival, and also as the ratio of D_0's.

Survival after ^{60}Co X-ray exposure was compared by using a dose rate of 150 rads/hr, based on a rounded value of 5.0 for the RBE; mice carrying 2-day-old tumors were then irradiated for the same length of time as with the pion beam, except for the 1000- and 1500-rad points, for which we used a dose rate of 500 rads/hr. Four mice per dose were used in control experiments, the cells were pooled after sacrifice, and then the procedure described before for dilutions and injections was followed.

Acute exposures with ^{60}Co γ-rays were made at a dose rate of 45 rads/min. Roentgens were converted to rads by use of a factor 0.96 (10, Table IV.1).

Acute exposures with 910-MeV ^{4}He ions were made at the 184-inch synchrocyclotron; in this case, the mice were enclosed in the same holder as in the pion experiment and irradiated one at a time. The average dose rate was about 275 rads/min.

The partial pressure of oxygen in vivo was measured with a Beckman pO_2 microelectrode. The microelectrode was inserted into the peritoneal cavity of mice carrying tumors of the same age as the irradiated ones, by means of an 18-gauge hollow needle. The microelectrode had been calibrated with mixtures of oxygen and nitrogen, and the calibration was linear from 21% down to 0.25% oxygen, the minimum concentration we could read on the maximum-sensitivity scale of the instrument. This is in the region of interest, since a sharp rise in radiosensitivity takes place for oxygen concentrations in the range of 0.2 to

0.6% (11). Normal mice and mice carrying from 1-day-old to 8-day-old lymphoma ascites tumors have been measured; 20 mice per point were used, and all measurements graphically recorded. Table I shows pO_2 values pertinent to this report. Changes in pO_2 between the second and third day (10^7 cells inoculum) are of particular interest because of levels of pO_2; between 2 and 5 mm Hg the radiosensitivity of ascites tumor cells is known to change rapidly (11).

RESULTS AND DISCUSSION

Results have been summarized in Table II along with the results obtained in our previous report (1).

Figure 3 shows the survival curve following irradiation with π^- at the Bragg peak. Although it may seem redundant, we have indicated the value of the extrapolation number as well as the value of the quasi-threshold dose, $\underline{D_Q}$, in all figures. In order to reach 0.1 level of survival, we have prolonged the line beyond experimental values, with the reasonable assumption that exponential survival would follow the three experimental points that fitted a straight line.

The small shoulder in this survival curve ($\underline{D_Q}$ = 44 rads) may be due to the low-LET components of the beam. The shoulder is much broader in the ^{60}Co γ-ray survival curve (Fig. 4, $\underline{D_Q}$ = 400 rads); this curve was obtained at a dose rate of 150 rads/hr, except the two highest doses, for which we used 500 rads/hr in order to keep the experiment within the same time limits as the pion experiment. This value of $\underline{D_Q}$, about 10 times as large as $\underline{D_Q}$ for π^-, seems to indicate faster recovery from irradiation with γ-rays.

Figure 5 shows the survival curve for acute irradiation with ^{60}Co γ-rays, at a dose rate of 45 rads/min; the extrapolation number (n = 1.5) is not significantly different from the value obtained with π^-. $\underline{D_Q}$ is only 1/5 that for low-dose-rate γ-rays, but almost twice as great as the value for π^- mesons.

Figure 6 shows the results obtained with 910-MeV ^{4}He ions. We have fitted the points to a straight line, but on the basis of this experiment it would be difficult to say whether or not the extrapolation number is 1.0. Consequently, we have written a question mark on Table II.

Figure 7 summarizes all these experiments, and it shows the limited range of doses covered in our π^--meson experiment.

With the acute irradiations (Figs. 5 and 6), the shoulders seem to be smaller. The fitting is not good enough to make comparisons with Berry's survival curves (12). He found that shoulders are present in the high-dose-rate curves and not in the low-dose-rate ones.

The RBE for pions has been estimated by comparing results obtained at a ^{60}Co dose rate equivalent to that of the pion beam, and also at a higher dose rate, as used in radiotherapy. In the first case, 0.1 survival was used; the RBE then was RBE = 5.4±0.6.

The assumption can be made that the $\underline{D_0}$ for the π^- should not change appreciably with dose rate. The RBE estimated on the basis of the $\underline{D_0}$'s was RBE = 2.9±0.5.

The OER cannot be estimated from survival curves obtained at different tumor ages (5, 7, 13, 14). In a 2-day-old tumor the cells are in the beginning of the log phase, and in a 5-day-old tumor they are entering the stationary phase. The fact that there is oxygen present in the 2- and 3-day-old tumors does not necessarily mean that the cells are fully oxygenated. This is indicated by the $\underline{D_0}$ value of 220 rads obtained with

with ^{60}Co γ-ray exposures. This value agrees with Belli and Andrew's results for 2-day-old ascites tumors (14).

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FIGURE LEGENDS

Fig. 1. Depth dose of π^- beam. Arrows indicate limits of position of mouse holder.

Fig. 2. Schematic setup for exposures of mice carrying 2-day-old ascites tumors.

Fig. 3. Survival of lymphoma ascites tumor cells after irradiation with negative pions in the Bragg peak as shown in Fig. 1. The dose rate was 25-30 rads/hr.

Fig. 4. Survival of lymphoma ascites tumor cells irradiated _in vivo_ with ^{60}Co γ-rays at a dose rate of 150 rads/hr. The two highest doses were obtained at a dose rate of 500 rads/hr.

Fig. 5. Survival of lymphoma ascites tumor cells exposed _in vivo_ with ^{60}Co γ-rays at a dose rate of 45 rads/min.

Fig. 6. Survival of lymphoma ascites tumor cells irradiated _in vivo_ with 910-MeV ^{4}He ions at a dose rate of 275 rads/min.

Fig. 7. Survival of 2-day-old lymphoma ascites tumors after low- and high-dose-rate irradiations with π^-, ^{60} γ-rays, and ^{4}He ions.

Table I. In vivo pO_2 measurements (mm Hg ± SD).

Tumor age (Ascites lymphoma)	Inoculum 10^6 cells	Inoculum 10^7 cells
2-day	9.0±3.0	7.2±3.0
3-day	5.4±2.8	3.2±1.6
5-day	1.0±1.0	<0.25
6-day	< 0.25	-------

Normal (no tumor): 41.2±9.1

Table II. Summary of results obtained on the survival of lymphoma ascites tumor cells after irradiation with different ionizing radiations.

Experiment No. and date	Radiation source and dose rate	0.1 survival (rads)	RBE (0.1 surv.)	Survival curve characteristics	RBE (Ratio of D_0's)
TWO-DAY-OLD LYMPHOMA ASCITES TUMORS					
22 6-27-68	π^- Bragg peak 25–30 rads/hr	220 (extended line intercept)	5.4±0.6	D_0 = 76±4 rads n = 1.8 D_Q = 44 rads	2.9±0.5
25-43 7-18-68 11-26-68	^{60}Co γ-rays 150–500 rads/hr	1180	≡ 1.0	D_0 = 350±40 rads n = 3.2 D_Q = 400 rads	
30 8-14-68	^{60}Co γ-rays 45 rads/min	600		D_0 = 220±30 rads n = 1.5 D_Q = 80 rads	≡ 1.0
24 7-15-68	^{4}He ions; 910 MeV (184-inch synchro-cyclotron) 275 rads/min	500		D_0 = 210±40 rads n = 1.0 (?)	
FIVE-DAY-OLD LYMPHOMA ASCITES TUMORS					
17 3-18-66	π^- Bragg peak 5.0 rads/hr	150	5.4±1.8	D_0 = 65±15 rads n = 1.0	5.4±1.8
35 7-7-66	^{60}Co γ-rays 5.0 and 12.5 rads/hr	806	≡ 1.0	D_0 = 350±50 rads n = 1.0	≡ 1.0

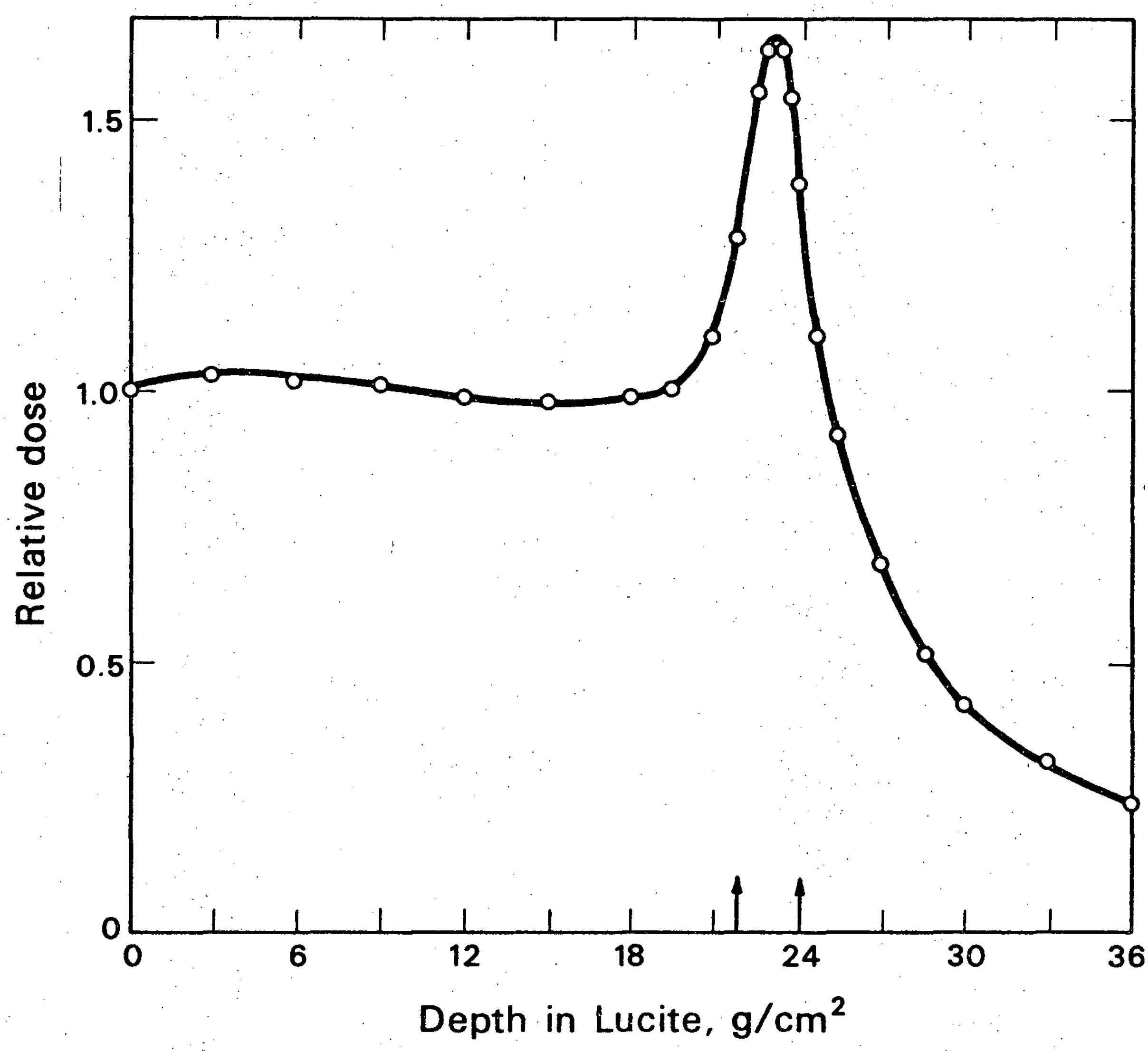

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Fig. 1

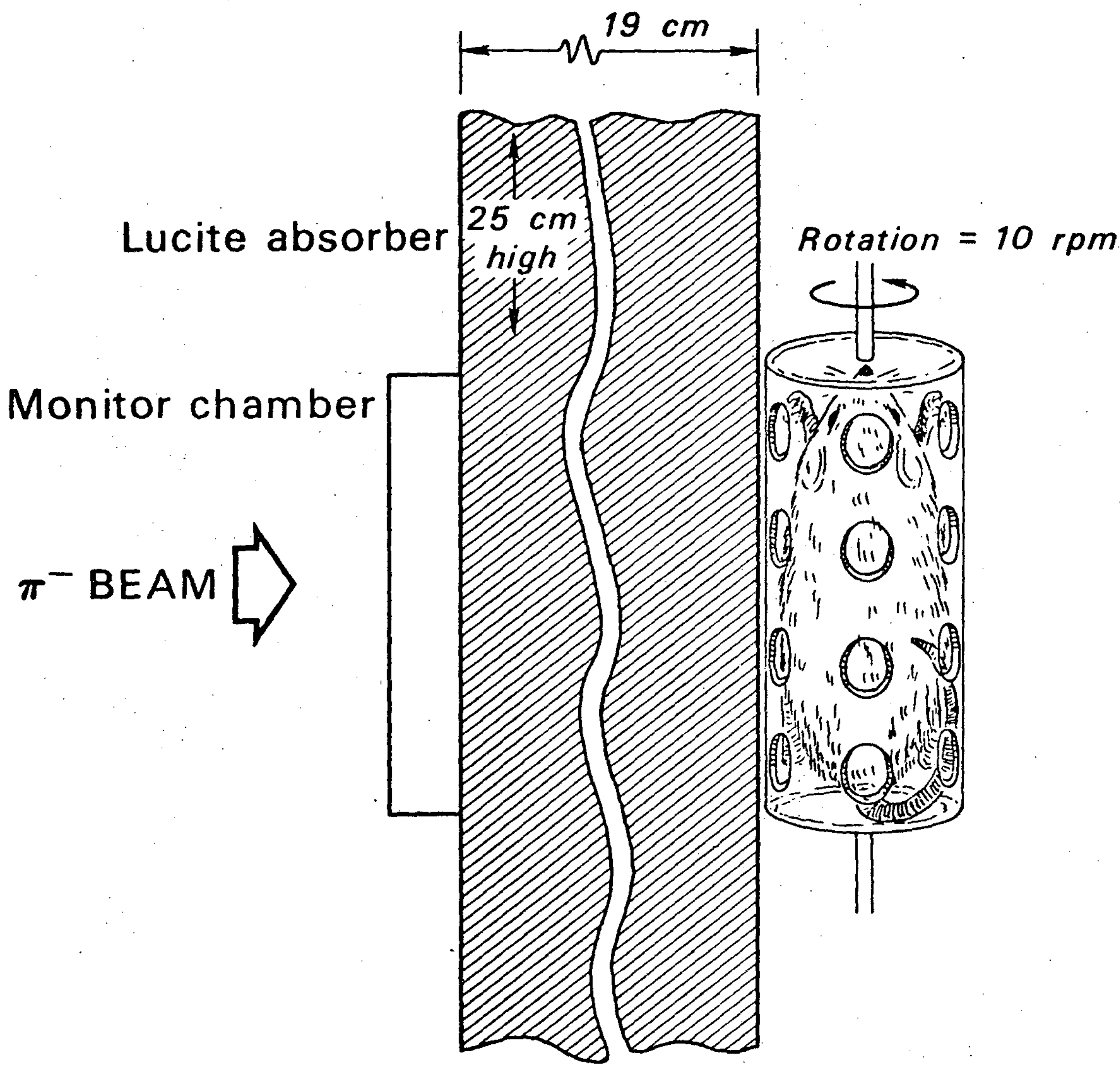

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Fig. 2

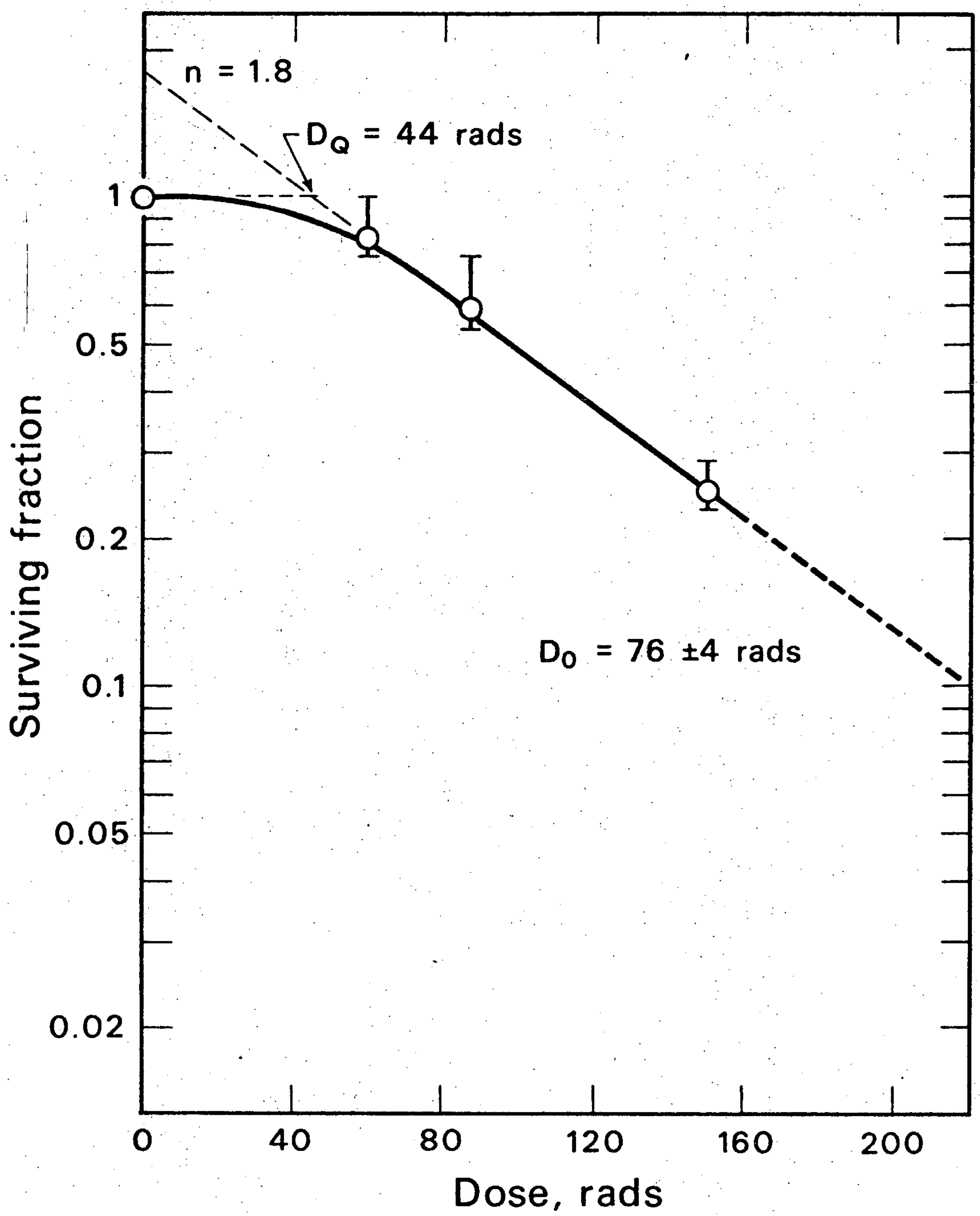

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Fig. 3

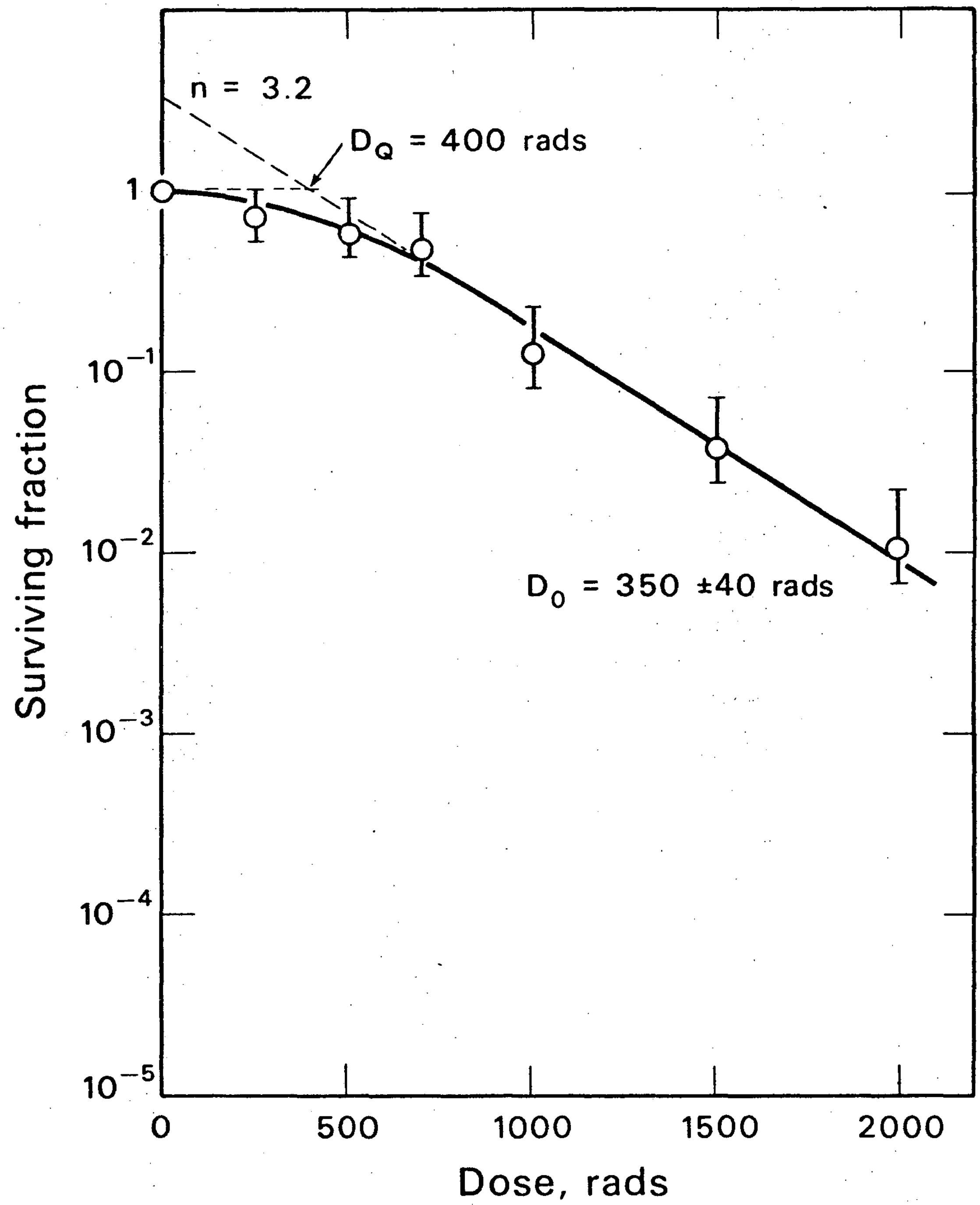

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Fig. 4

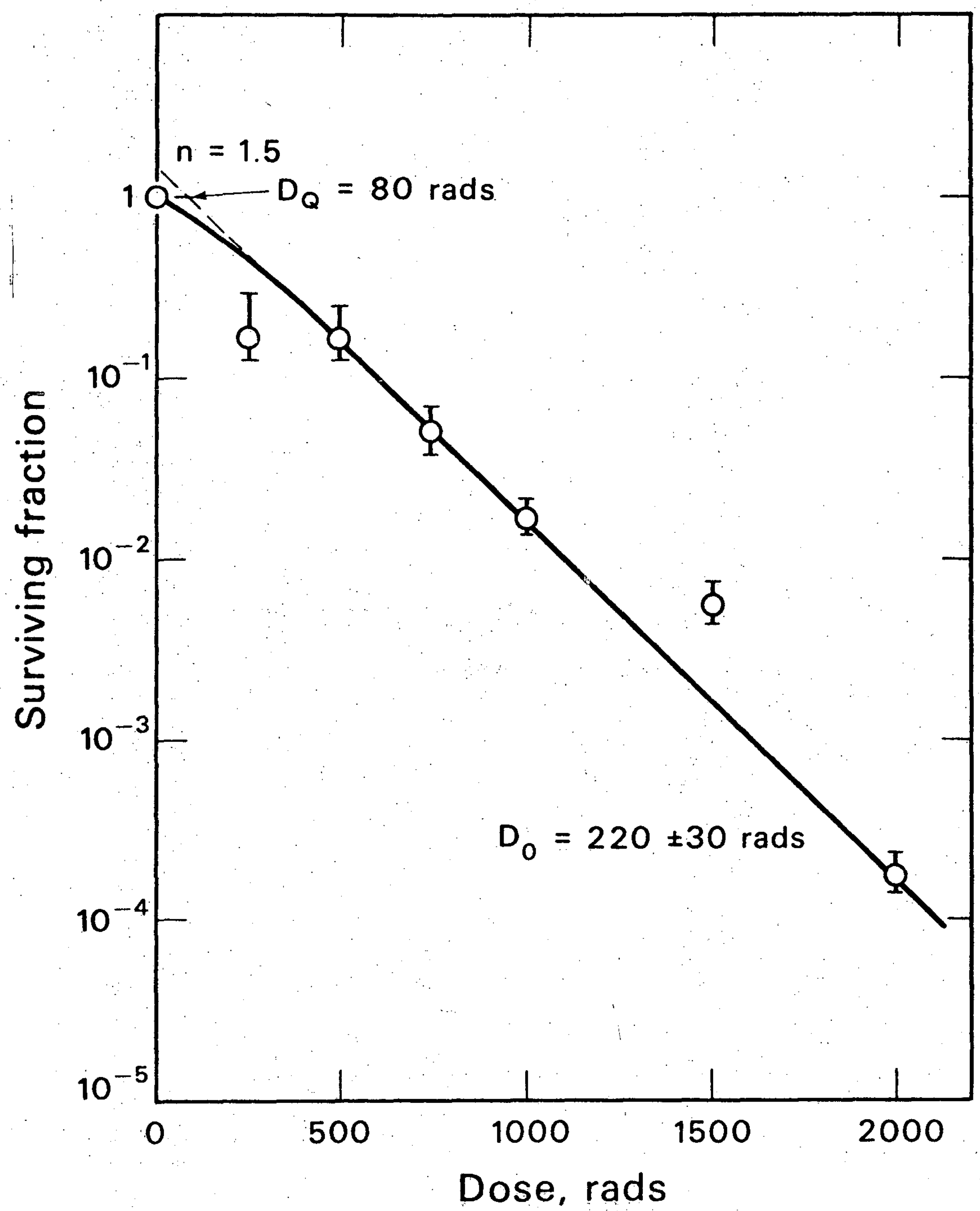

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Fig. 5

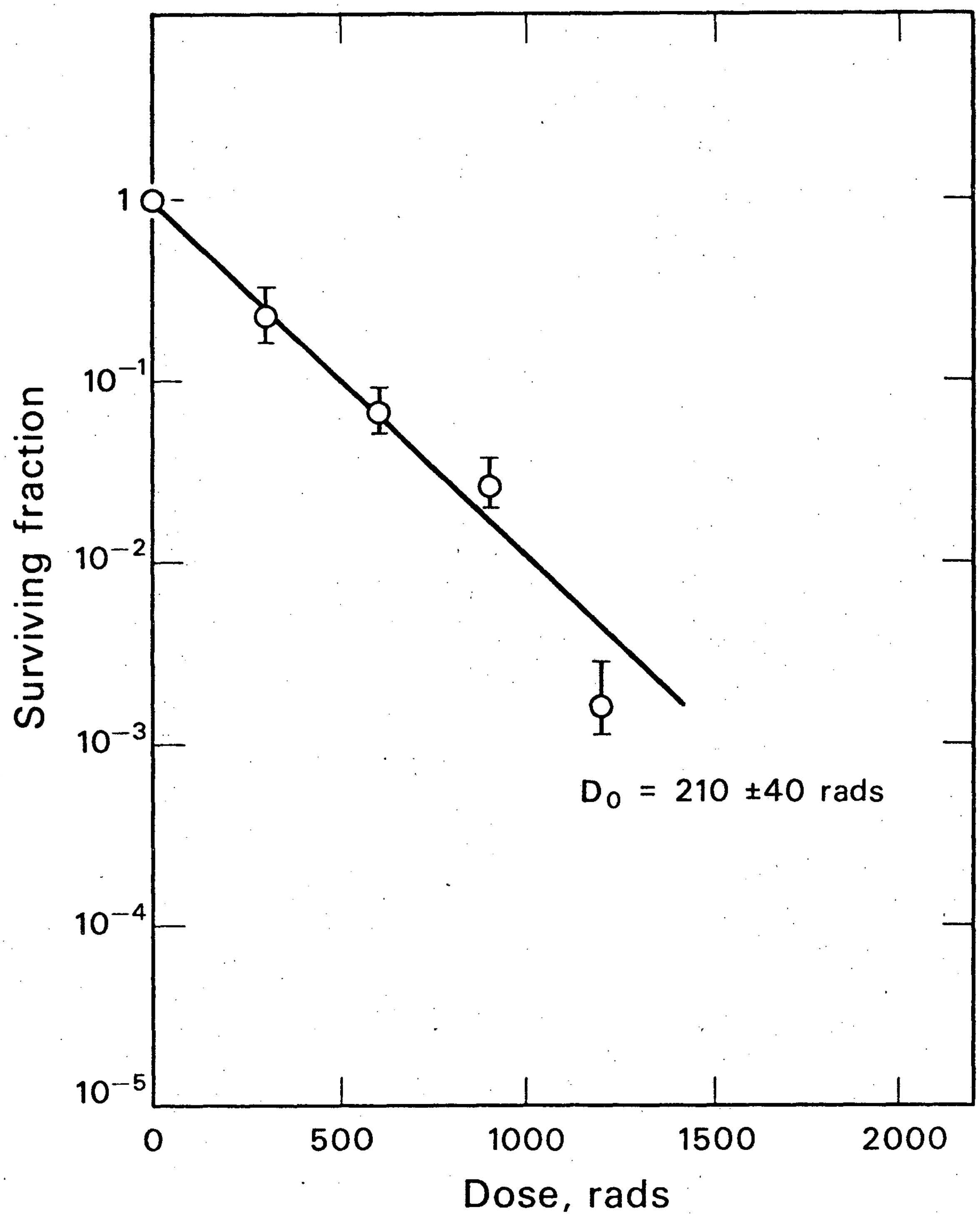

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Fig. 6

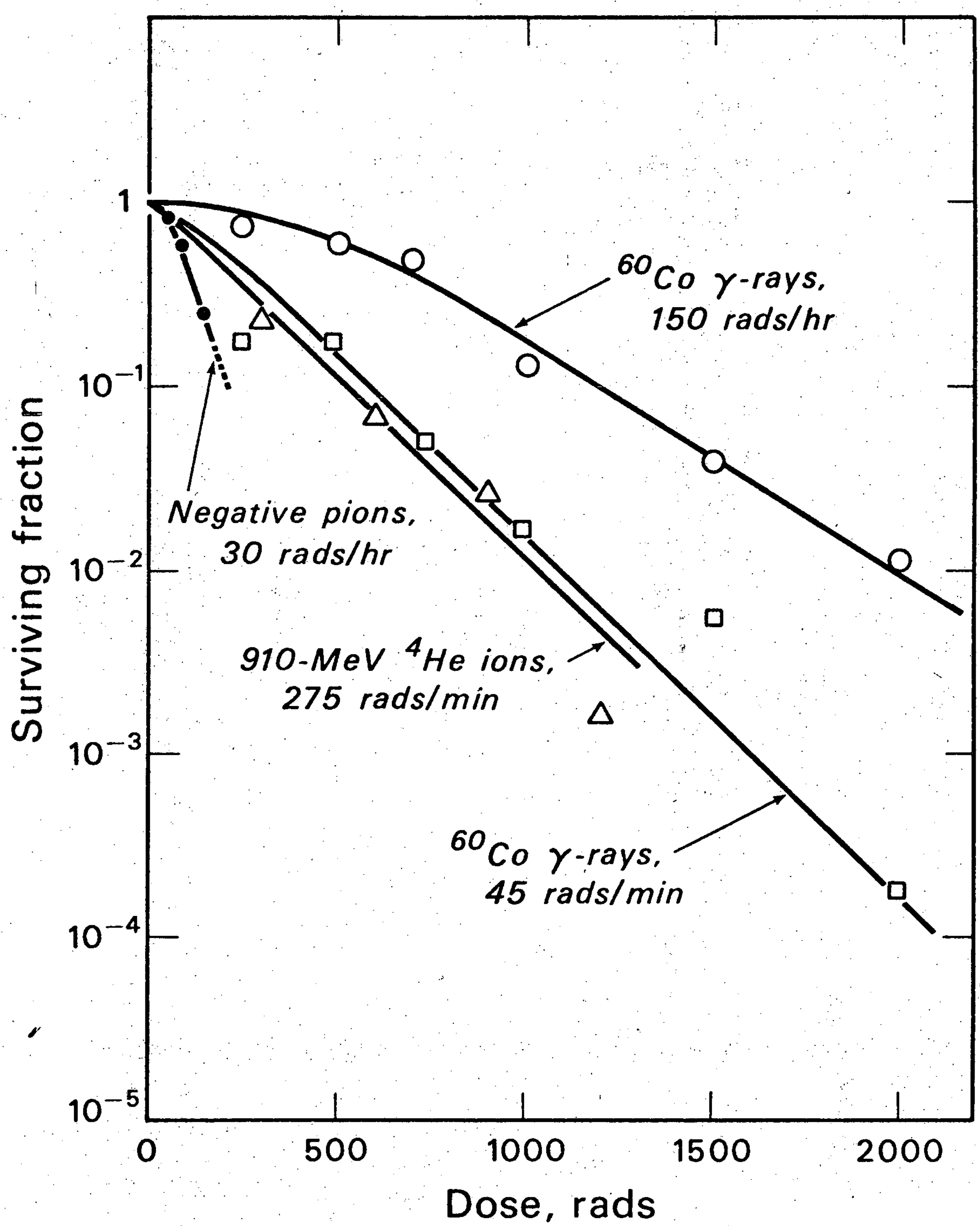

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Fig. 7

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