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EFFECT OF NEGATIVE PIONS ON THE PROLIFERATIVE CAPACITY OF ASCITES TUMOR CELLS (LYMPHOMA) GROWN IN VIVO[1]

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J. M. FEOLA, C. RICHMAN, M. R. RAJU, S. B. CURTIS, and J. H. LAWRENCE, Effect of negative pions on the proliferative capacity of ascites tumor cells (lymphoma) *in vivo*. *Radiation Res.* ___, pp. ___-___, ____.

ABSTRACT

We have attempted to determine the relative biological effectiveness (RBE) of negative pions in the Bragg-peak region as compared to the plateau region and to gamma rays. We irradiated LAF_1 mice, bearing 5-day-old lymphoma ascites tumors, in the peak and plateau regions of a 90-MeV pion beam for 40 hours in temperature-controlled holders. The animals were then sacrificed; lymphoma cells were withdrawn and titrated into adult female LAF_1 mice. The proliferative capacity of the irradiated tumor cells was evaluated after 8 weeks by observing the percentage of animals developing ascites tumors. Surviving fractions were then calculated from LD_{50}'s of control and irradiated animals. Radiation doses in the plateau region were measured with LiF dosimeters calibrated against cobalt-60 gamma rays. We calculated peak doses from those at the plateau, using a measured average peak-to-plateau ionization ratio of 1.5. Doses in the plateau region ranged from 145 to 250 rads; doses in the peak region ranged from 220 to 380 rads. The survival curve for cells irradiated in the peak region gave a D_0 of 65 ± 15 rads. The plateau points were not reliable. A replicate experiment was performed using Co^{60} γ-rays, yielding a survival-curve D_0 of 350 ± 50 rads. If the γ-ray D_0 is taken as a baseline, an RBE of 5.4 ± 1.8 is obtained for negative pions in the peak region, based on the ratio of peak-region D_0 to Co^{60} D_0.

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KEY WORDS: RBE
Pion
Meson
Mammalian
Ascites
Lymphoma

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INTRODUCTION

It has been suggested[3,4] (1-3) that negative pions (π^- mesons) might have applications in radiotherapy if a beam of sufficient intensity could be made available. Negative pions have the unusual property of being captured by atomic nuclei when they come to rest in matter. In tissue, pion capture by the light elements (carbon, nitrogen, and oxygen) results in nuclear disintegration and a yield of short-range but highly ionizing charged particles, mostly α-particles and protons. This additional energy enhances the high ionization density produced by the Bragg peak at the end of a charged particle's range. Furthermore, by choosing pion energy properly, we can stop pions at a preselected distance within the volume to be treated. In the following discussion, the initial low-ionizing portion of the pion's path will be called the "plateau" region and the increased ionization at the end of the pion's range will be called the "peak" region.

Studies of the therapeutic possibilities of negative pions have been carried out in this Laboratory[3] (3). The results indicate that further investigations, of increased refinement, should be made. The proliferative capacity of murine lymphoma cells grown and irradiated *in vivo* and *in vitro* has been investigated in this Laboratory for a number of years with X-rays, α-particles, and various heavy ions, under various conditions of oxygenation[5] (4). The study of this system has progressed to a stage where an attempt can be made to estimate quantitatively the effects of pions in tissue despite the low dose rates now available. Initial work in this Laboratory,[6,7] on the lymphoma cell system in mice, demonstrated

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a peak-plateau difference in the cytological effects produced by negative-pion beams. These experiments were later extended[8] using a plant-cell system. Vicia faba roots were exposed to a negative-pion beam, and significant peak-to-plateau differences were found for growth rate of the roots after irradiation, for anaphase abnormalities, and for cells containing micronuclei. These results, coupled with improved dosimetry (5), stimulated the experiments reported here.

MATERIALS AND METHODS

Four types of experiments were performed: A) Pion effects in vivo with environmental control; B) Co^{60} replication of Experiment A; C) Pion effects in vivo without environmental control; and D) Acute X-ray irradiation in vivo.

Experiment A: Pion effects in vivo with environmental control.

Full descriptions of the apparatus associated with the 184-inch synchrocyclotron, and methods of using it to produce the 90-MeV pion beam, have already been reported[3,4] (3). The beam-transport system consists of a small quadrupole focusing magnet, a beam-bending magnet, and a large quadrupole focusing magnet that delivers the beam from the cyclotron tank to the meson cave. In these experiments, the final focusing magnet was made part of a second shielding wall placed in the cave to protect the counting equipment from ambient neutrons. The complete experimental setup is shown in Fig. 1.

Two wooden boxes with Lucite ends provided environmental control, one for the mice to be irradiated in the beam, and the other for control mice. The temperature of the air circulating through the boxes was continuously recorded, and kept at 21.5±0.5°C by a thermostat with heater and blowers.

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The mice were placed in Lucite holders, each built to hold 8 mice. The dimensions of the holders were 54X98X106 mm; the mice were placed in cylindrical holes 24 mm in diameter and 75 mm long, with small lateral holes for ventilation. One group of 8 mice was placed in the plateau region of the negative-pion beam, one group in the peak region, and one group was used as a control. The mice were of the LAF_1 strain, 15 weeks old, from Jackson Laboratory, Bar Harbor, Maine. They were fed wet food during irradiation and had four equally spaced 1-hour rest periods for drying, eating and drinking at will. Figure 2 is a photograph of the arrangement inside the temperature-controlled irradiation box. The negative-pion beam entered from the right and passed first through the gas ionization-chamber monitor (96% argon + 4% CO_2), then through 3 inches of Lucite absorber and holder to the "plateau" mice. After passing through 4 more inches of Lucite, the beam entered the "peak" holder. This thickness was determined with a lithium-drifted silicon detector.[9] The Jordan dosimeter was placed behind 1/2 inch of Lucite adjacent to the last holder and served as a second monitor and dose-rate meter. This monitor was frequently checked from the control room via closed-circuit television. The holder with the control mice is also shown in Fig. 2. In the actual experiment, this holder was in a separate, temperature-controlled box away from the beam.

Five days before beginning irradiation, all the mice were injected intraperitoneally with L#2 lymphoma cells, whose tumor-forming ability has not changed in the last three years.

The total irradiation time, excluding the 1-hour rest periods, was 40 hours. At the end of the irradiation, the animals were sacrificed

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and peritoneal ascites fluid with lymphoma cells was withdrawn. In this part of the study, five plateau-region mice and five peak-region mice were used. The cells were counted and injected intraperitoneally at various dilutions into 710 healthy adult female LAF_1 recipient mice. Cells from four control mice were pooled from two groups of two animals each, and handled in the same way as the experimental mice. The remaining experimental mice were used for cytological studies (6). The recipient mice were divided into groups of ten animals each, and each member of a group received an identical injection. Each group, however, received a different concentration of cells, we evaluated the cells' proliferative capacity at the end of 8 weeks by noting the percentage of animals developing ascitic tumors in each group. The LD_{50} (the number of cells necessary to produce tumors in 50% of the animals) and 95%-confidence intervals were calculated by the method of Litchfield and Wilcoxon (7). Finally, surviving fractions were calculated by forming the ratio of LD_{50} of the control mice to that of each of the irradiated mice.

Radiation doses were measured with LiF dosimeters (Co^{60} calibration), distributed in front and in back of the mouse holder in the plateau region. Since the LET of the pions in the plateau region is less than 1 keV/μ, LiF dosimetry was applicable. The situation in the peak region was more complicated[9] (5). Here the pion beam was a mixture of high- and low-LET radiations. Peak-region doses were calculated from those in the plateau region, using an average peak-to-plateau ionization ratio of 1.5. This ratio was estimated from measurements made through the peak and plateau regions with a silicon detector. Some uncertainties in

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the measurement still exist and are being investigated, but we believe that ionization in the peak region is adequately measured by the silicon detector. The total doses in the plateau region ranged from 145 to 250 rads, and the doses in the peak region ranged from 220 to 380 rads.

Experiment B: Co^{60} replication of Experiment A.

Since it is difficult to perform a parallel experiment with X-rays because of the long irradiation times required, an experiment was done using Co^{60} gamma rays. Two dose rates were used: 5 and 12.5 rad/hr. The animals, all bearing 5-day-old tumors at the beginning of the experiment, were kept in the holders for the same length of time as in the pion experiment to keep the stress the same. The irradiation times varied with the dose used. Four animals were used as controls, and two were irradiated at each of the following doses: 100, 150, 200, 300, 400, and 500 rads. The irradiated lymphoma cells were injected into 410 female LAF_1 mice at various dilutions, and the surviving fractions were determined as already described.

Experiment C: Pion effects in vivo without environmental control.

This experiment, preliminary to Experiment A and without environmental control, was designed to detect the effect of hypoxia (8) on the surviving fraction of cells irradiated in vivo in the plateau and peak regions. Four mice were exposed in each pion-beam region; two had 3-day-old tumors (supposedly well-oxygenated), and two had 7-day-old tumors (supposedly more hypoxic). The irradiated cells were injected into 510 LAF_1 female mice at various dilutions as described above.

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Experiment D: Acute X-ray irradiation in vivo.

In order to compare the effect of acute X-ray irradiation with "chronic" Co^{60} irradiation, animals bearing 7-day-old tumors were irradiated with 220 kV X-rays (HVL = 1.4 mm Cu) at a dose rate of 150 rads/min. Doses of 100, 250, 400, 500, and 750 rads were given, and the procedure described above was followed; 350 female LAF_1 mice were injected at various dilutions.

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RESULTS

Experiments A and B.

The resulting surviving fractions in the peak and plateau regions and in the low-dose-rate Co^{60} experiment are given in Fig. 3. Inconsistent data from the plateau-region irradiation prevented a reliable determination of dose dependence of the surviving fraction in this region. The peak region and Co^{60} data yielded sufficiently consistent results so that estimations of D_0 could be made. (D_0 is called the mean lethal dose and it is the dose in rads required to reduce the proportion of surviving cells from 1 to 0.37 in the exponential region of survival curves). The computed D_0 values are:

$$D_0(\text{peak}) = 65 \pm 15 \text{ rads}; \qquad D_0(Co^{60}) = 350 \pm 50 \text{ rads}.$$

The RBE for the π^- mesons at peak based on these values is:

$$\text{RBE} = \frac{D_0(Co^{60})}{D_0(\text{Pk})} = 5.4 \pm 1.8 .$$

Experiment C.

Unpublished data obtained in this laboratory indicate that the lack of environmental control in this experiment would make quantitative evaluations somewhat unreliable. It was found that there was no significant difference between results from 3-day-old ("normal") and 7-day-old (relatively hypoxic) tumors. On the other hand, a significant difference between the surviving fractions obtained from pion irradiation in the peak and plateau regions encouraged the design of the environmentally controlled Experiment A.

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Experiment D.

The value obtained for D_0 in acute X-ray irradiation *in vivo* is 280±50 rads. Comparison of this value with acute X-ray *in vitro* values of 105±15 rads for the hyperoxic case and 375±45 rads for the anoxic case, recently measured in this laboratory,[10] indicates the *in vivo* 7-day-old tumor cells are considerably less well-oxygenated than the hyperoxic *in vitro* cells. In fact, the *in vivo* D_0 lies closer to the *in vitro* anoxic D_0 than the average of the hyperoxic and anoxic values (240 rads). These results are consistent with the observation that tumors, especially well-advanced ones, contain poorly oxygenated cells.

DISCUSSION

The study of the action of negative pions on mammalian cells grown *in vivo* is difficult to carry out due to the low dose-rates available at present. In the pion plateau region, the average dose-rate of 5 rads per hour in this experiment requires long irradiation times to cause measurable biological damage. For long irradiation episodes, the animals must be confined and therefore are under stress. During irradiation, the cell population undergoes many poorly understood dynamic processes and a certain amount of recovery and repair. Technical problems in keeping a biological system anoxic for the required time make pion-beam study of the oxygen effect extremely difficult.

Despite the uncertainties arising from these uncontrollable factors, the results give a clear indication that the peak region of the pion beam is more effective than the plateau region in inhibiting the proliferative capacity of these lymphoma cells. In addition, peak-region irradiation inhibits

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the proliferative capacity significantly more than low-dose-rate Co^{60} gamma rays do. The ratio of D_0's from the Co^{60} data and from the pion peak-region data gives an RBE of 5.4±1.8 in the peak region relative to gamma rays. The size of the error here indicates that considerable uncertainty exists in the determination of the D_0's, as is seen in Fig. 3. If an RBE of 0.8 is assumed for gamma rays relative to X-rays, as has been reported (9, 10, 11), the RBE becomes 4.3±1.8 relative to X-rays.

In evaluating this type of radiation for cancer therapy, we note that the RBE measured at such low dose-rates need not equal the RBE at the necessarily higher therapeutic dose-rates. The damage caused by the low-LET Co^{60} gamma rays will probably be considerably more repairable than the damage caused by the high-LET components of the pion beam in the peak region. Recovery from gamma irradiation during a low-dose-rate experiment would manifest itself by increasing the gamma-ray D_0. This would increase the RBE over that expected in higher-dose-rate experiments when there is less time for repair. Thus, the D_0 ratio, which is RBE, would decrease.

Although the results in the plateau region are inconclusive because of the inconsistency of the data, the average LET in this region is expected to be between 0.2 and 0.3 keV/μ and so should be no more effective than X- or gamma rays in causing biological damage. An experiment must be performed to substantiate this, however, Loughman has indicated this may be true for certain nonlethal radiation effects (6).

In conclusion, we have shown that the peak region of a pion beam is considerably more effective than an equivalent dose of gamma rays in inhibiting the proliferative capacity of lymphoma cells *in vivo*. This

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result implies that there is a biologically significant component of high-LET radiation in the peak region of a pion beam, as predicted by calculation (2,12). This in turn implies that the oxygen-enhancement ratio will be low, making the peak-region radiation of a pion beam more effective than conventional radiation in killing the anoxic cells found in tumors (13). More experimentation is indicated before the evaluation of this new type of radiation is decisive, but it appears at present that negative pions have interesting possibilities in tumor radiotherapy.

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FOOTNOTES

1. This work done under auspices of the U. S. Atomic Energy Commission, American Cancer Society, and Office of Naval Research.
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FIGURE CAPTIONS

Fig. 1. Schematic view of apparatus for production and focusing of the π^- beam. The position of the ionization chamber, Jordan dosimeter, and mice holders can be seen in the meson cave.

Fig. 2. Photograph showing the arrangement inside the temperature-controlled irradiation box.

Fig. 3. Survival curves of lymphoma cells irradiated *in vivo* with a beam of negative pions and with Co^{60} γ-rays under similar conditions. Circles show plateau points.

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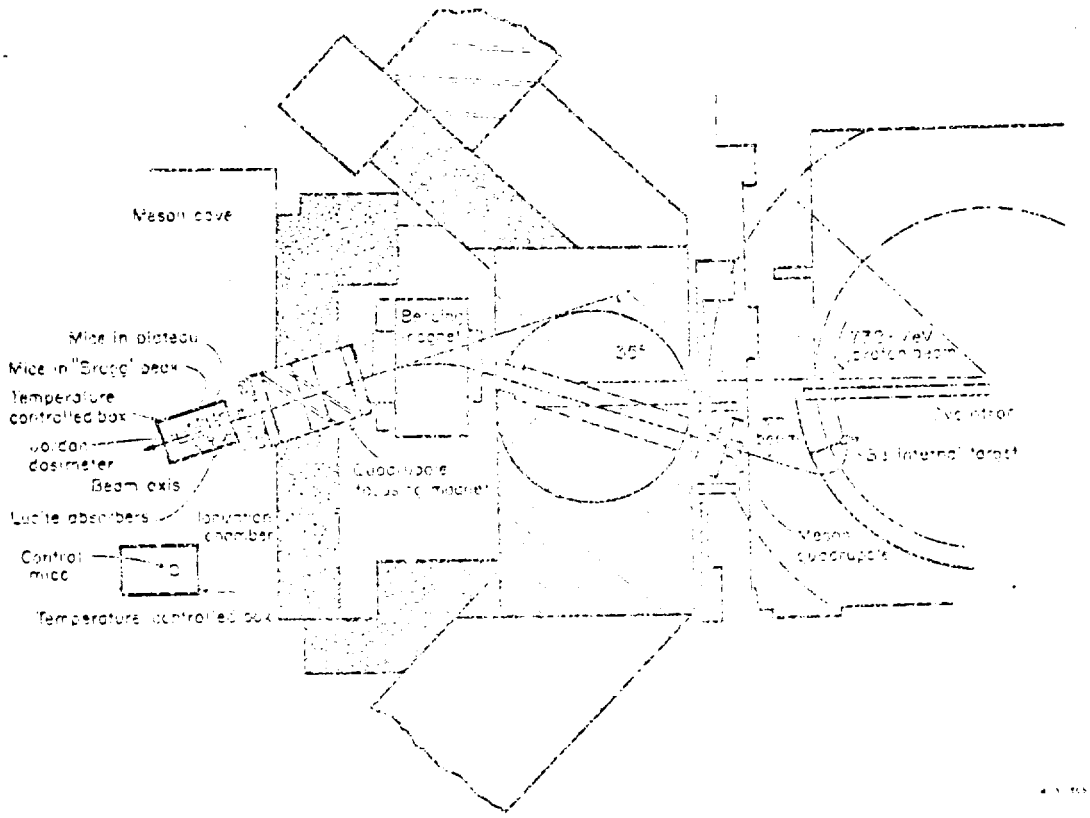
Meson cave
Mice in plateau
Mice in "Bragg" box
Temperature controlled box
dosimeter
Beam axis
Lucite absorbers
Ionization chamber
Control mice
Temperature controlled box
35°

Fig. 1

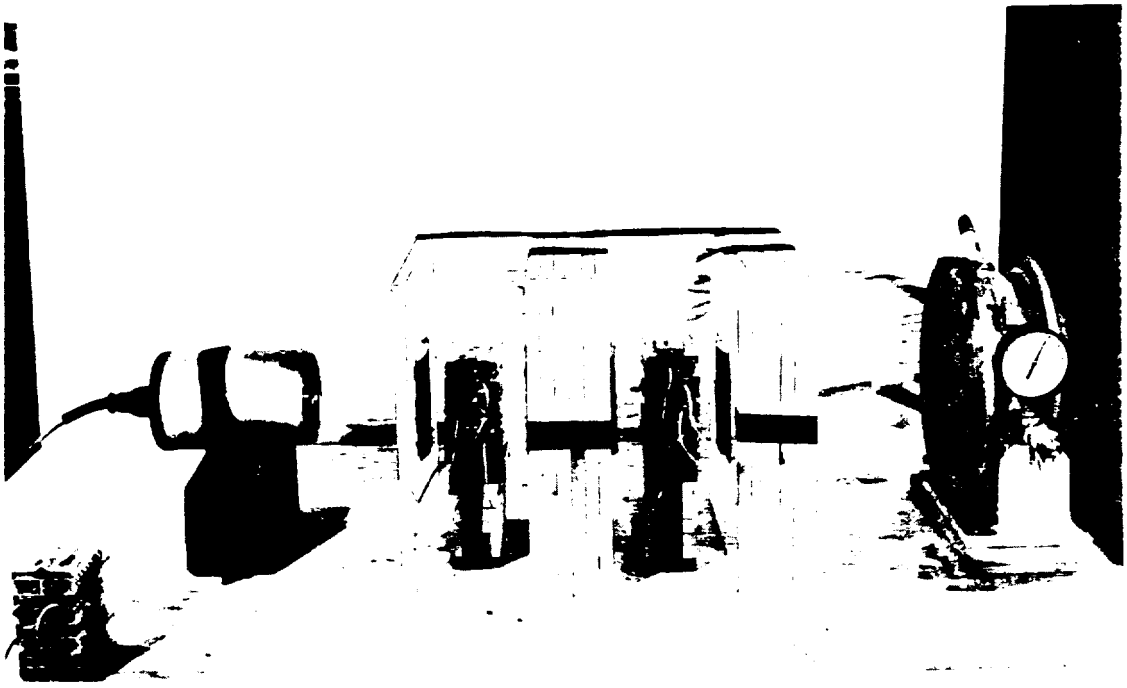

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Fig. 2

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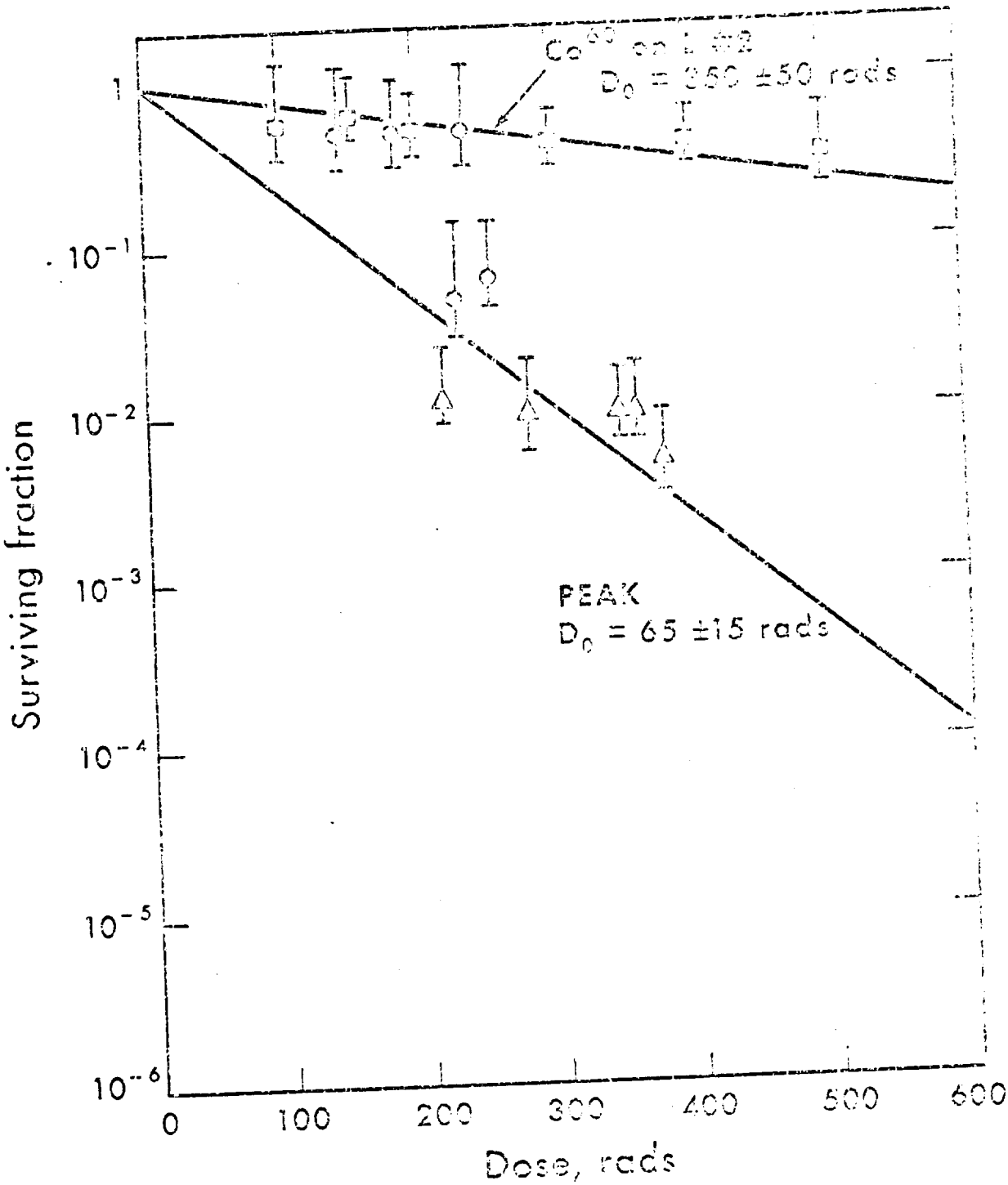

Fig. 3

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