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High-Let Radiation Carcinogenesis

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ABSTRACT

The dose-response curves for the induction of tumors by high-LET radiation are complex and are insufficiently understood. There is no model or formulation to describe the dose-response relationship over a range 0-100 rad. Evidence suggests that at doses below 20 rad the response is linear, at least for life shortening and some tumor systems. Thus, limiting values of RBEs for the induction of cancer in various tissues can be determined, but it will require sufficient data obtained at low single doses or with small fractions.

The results obtained from experiments with heavy ions indicate an initial linear response with a plateauing of the curve at a tumor incidence level that is dependent on the type of tissue. The RBE values for the heavy ions using ^{60}Co gamma rays as the reference radiation increase with the estimated LET from 4 for ^{4}H to about 27 for ^{56}Fe, ^{40}Ar. The dose-responses and RBEs for ^{56}Fe and ^{40}Ar are similar to those for fission neutrons. These findings suggest the possibility that the effectiveness for tumor induction reaches a maximum.

INTRODUCTION

The studies of high-LET radiation carcinogenesis have two aims that are separate but are, nevertheless, interrelated. The aims are, first, the provision of quantitative dose-response relationships that can be used in the estimate of risks, and second, to exploit the radiation quality dependent differences in energy deposition and relate them to the processes of tumor induction. The role of experimental studies of risk estimates has become more important as the hopes of estimates based on human data wane as a result of the new dosimetry of the exposures to survivors at Hiroshima (1).

The nature of the energy deposition in the target cells that is dependent on the radiation quality or track structure determines the probability of the target involved in cancer induction being hit. Recently various monoenergetic heavy ion beams have become available thus increasing the armamentarium for probing the relationship of dose distribution and cancer induction. It is not yet clear whether or not the extremely difficult task of linking the changes induced by deposition of energy and cancer induction can be made in the detail required for modeling of mechanisms and the understanding of the dose-response relationships but the goal is worthy of pursuit.

In this paper we discuss some of the current information about neutron radiation carcinogenesis and report new results for heavy ion carcinogenesis.

Neutron Radiation Carcinogenesis

We are now in the 5th decade of high-LET external radiation carcinogenesis studies. For many of those years interest in this facet of radiation induction of cancer was limited and intermittent, perhaps due to the lack of suitable facilities dedicated to biological research and also the

lack of pressure from those responsible for protection standards. In the 1950's and 1960's not many people were exposed to neutrons so concern about high-LET radiation was concentrated on the internal emitters.

The understanding of neutron carcinogenesis and life shortening, which at low doses is largely caused by excess cancer, has increased steadily if slowly (2,3). The progress has depended on the development of suitable facilities and the slow process of learning how to design and carry out critical experiments, and of equal importance, how to analyze appropriately the data. That learning process continues.

The early studies (4-7) established the greater effectiveness of neutrons for the induction of tumors, life shortening and other tissue effects than low-LET radiation. It was also suggested that the relative effects of small daily doses of fast neutrons as compared with gamma radiation was greater the more the radiations were protracted.

Later studies have been concerned with dose-response relationships and RBEs for life shortening and specific tumors, additivity and dependence on energy (see reference 2). Despite considerable success in carrying out fairly large and complex experiments with ever decreasing doses and dose rates some fundamental questions remained unanswered. For example, there is, as yet, no acceptable model that describes the dose-response relationships for either life shortening or cancer induction over a reasonable range of doses (0 to 100 rad). It is becoming increasingly clear from the experimental data, especially for life shortening, that the initial part of the dose-response curve is linear, perhaps up to about 20 rad, and the curves then bend over (convex up). The bending over appears to occur at different dose levels for different tumor types but in all those reported the bending

over is obvious above about 25 rads. While there has been increasing agreement that the dose-response for life shortening, at low doses (< 20 rads) is linear (8,9), there is as yet no data for tumor induction by neutrons that is adequate to indicate whether a linear or a square root of the dose fit is correct (10). In the case of Argon-40 a linear fit is clearly more appropriate than a square root of the dose fit over the range of 0-20 rad (see Fig. 4, the details of the experiment and analysis are to be published elsewhere). The lesson from all these studies is that the estimate of risks at very low doses, whether directly or by interpolation, must be based on data obtained for a range of doses below 15-20 rad - no trivial task.

Linear dose responses and additivity have been assumed to hold for the purposes of risk estimation and protection standards concerning high-LET radiations. The lack of additivity, and the increased effect of fractionation, at least with total doses in excess of 20-40 rads, became clear, initially, from life shortening experiments (11-13). A number of studies on tumor induction and in vitro transformation have suggested that protraction or fractionation of the neutron dose can increase the effect in striking contrast to the reduction in effect found with low-LET radiation (10,14-16). It is important that the question of additivity at low doses per fraction and low total doses, especially at low dose rates be resolved unequivocally.

The hope is that sufficient data for the dose-response relationships of a representative spectrum of tumors for use in establishing risk estimation directly will be obtained. The use of such data for protection standards depends on the establishment and acceptance of some method of extrapolation

of effects or risks from experimental animal data to humans.

The lack of accepted methods of extrapolation across species has certainly not helped the proponents of discarding the use of RBEs. There is a case, although not well documented, that could be made for the extrapolation of RBE values based on the assumption that the factors influencing the relative effectiveness are more likely to hold across species than the quantitative values of the effects of the individual radiation qualities.

Figure 1 illustrates schematically some of the problems that the use of RBE presents. RBE values for tumorigenesis are dependent on dose, dose rate, dose fractionation, LET, neutron energy and are tissue dependent.

A major question is at what dose levels of both neutrons and the reference radiation the effect per rad and the RBE become constant for the various tissues. In the case of life shortening, Storer and Mitchell (8) found limiting values for RBE at doses below 20 rad. However, in the case of tumors, largely because of the extreme range of initial slopes for the responses to low-LET radiation, values range from $1-\infty$.

It is important to note that the problem of distinguishing between advancement of time of appearance from an increase in the number of tumor bearing animals has not been solved completely. If advancement of time of appearance is the radiation-induced change the method of analysis must be appropriate for assessing such a change (17), and in the case of RBE be carried out also for the reference radiation.

Heavy Ion Studies

There are a number of reasons for studies of heavy ion radiation carcinogenesis. The introduction of heavy ions, for radiotherapy and the

likelihood of an increasing number of persons in space, where exposure to heavy ions can occur, makes risks estimate for heavy ion effects desirable.

The relationship of RBE to LET has been determined for a number of biological effects (18-23) but not for cancer induction. Despite the problems with determination of meaningful LET values and the question of their appropriateness it is important to have information about the RBE-LET relationship for tumor induction. Perhaps more interesting will be correlations of the tumorigenic effect of high-LET radiations and the particle track structure.

MATERIALS AND METHODS

Radiation Procedures

The exposures to heavy ions were carried out at the Bevalac, (Lawrence Berkeley Laboratory (LBL),in the case of Carbon-12, Neon-20, Argon-40 and Iron-56 and exposures to Helium-4 were at the 184 in synchrocyclotron at LBL. The BEVALAC accelerator and the dosimetry employed have been described elsewhere (24).

Since we wished to determine the carcinogenic effect of beams varying over a wide range of LET's it was necessary to use spread Bragg peaks. The plateau portion of the beams are the most desirable because fragmentation is reduced to a minimum. However, with the ions available when we embarked on this experiment it was not possible to obtain plateau beams with a sufficient spread in LET's. It was considered the narrow width of the Bragg-peaks made them unsuitable for the dose distribution required in the tissues under study. The Bragg peaks were spread with a rotating spiral ridge filter of brass. The ^{4}He, ^{12}C and ^{20}Ne Bragg peaks were

spread to 10 cm and ^{40}Ar peak to a 4 cm configuration.

The iron beam became available after we had designed the original experiment and exposures to ^{56}Fe were carried out in the plateau of the beam. The positions of the mice in the various beams is shown in Fig. 2.

The exposures to all beams were partial body with the head and thorax in the field. The discreteness of the beam was reflected in the absence of effects on the exquisitely sensitive ovary. The reference radiation was high dose rate (42-58 R/min) ^{60}Co gamma radiation, and we used the same partial body exposure as for the heavy ions was used. The abdomen and posterior portion of the mice were shielded with 1.2 cms of lead. In the case of the highest dose levels the shielding was insufficient to protect the ovary completely.

Experimental Test System

The tumorigenic effect of the heavy ion beams has been assessed on the Harderian gland of $B6CF_1$/Anl (C57BL/6J/Anl X BALB/cJ/Anl) Female 100-120 day-old mice. About 2 weeks prior to irradiation 2 pituitaries from other $B6CF_1$ mice were put in the spleen of the hosts. These pituitary isografts provide a source of pituitary hormones that are beyond the inhibitory control of the hypothalamus. Assessment of the secretory activity of the grafts has been made in various ways: 1) Determination of the estrus cycle. The active grafts induce a temporary suppression of the normal cycle. 2) Determination of serum prolactin levels, and 3) Macro and microscopic assessment of mammary gland activity at time of sacrifice. The reason for using the pituitary isografts is that they enhance markedly the expression of radiation-induced Harderian gland tumors and advance the time of appearance.

The grafts change an experimental tumor system that is moderately susceptible into one that is markedly susceptible. The pituitary hormone-induced advance in time of appearance decreases the duration of the experiment.

The results are based on prevalence at sacrifice at about 600 days of age. The time of sacrifice has been chosen in an attempt to reduce problems of competing risks that increase with time but at a time when all tumors that will occur have started to develop. While the majority of tumors were detected macroscopically some were detected only by microscopic examination of the glands. All tumor types were included in the determination of prevalences. The assignment of the mice to the various experimenal groups is shown in Table 1.

RESULTS

A report of the preliminary results has been made previously (25). The current results are shown in Figure 3. It can be seen that the tumorigenic effect of all the heavy ions was markedly greater than the effects of the reference radiation. The initial slopes of the curves increase from the shallow slope of ^{60}Co gamma radiation to the steep slope of the ^{56}Fe and ^{40}Ar response. The curves for ^{12}C, ^{20}Ne, ^{56}Fe and ^{40}Ar bend over and in general at lower dose levels for the higher LET beams. The difference in prevalence of tumors induced by the heavy ions becomes less with increasing dose. After exposure to 700 rad of ^{60}Co gamma and ^{4}He radiation the prevalence reached 40-45% comparable to the higher LET radiation results shown here. The level at which the response curves appear to plateau is assumed to be influenced by a number of factors, including the susceptibility of the mouse population to the tumor under study and inactivation of potential tumor cells. The dose at which the curve

plateaus must involve other factors, for example, fluence and particle track structure.

In Figure 4 the initial part of the dose-response curves for ^{56}Fe and ^{40}Ar are compared with the slope of the curve for the response to JANUS fission neutrons. The neutron data are based on a life-span study rather than from single-time prevalence data.

It appears that the initial slopes and the shapes of the dose-response curves for exposures to the Fn ^{40}Ar and ^{56}Fe are similar. These results suggest the possibility that the carcinogenic effect, as a function of LET, reaches a maximum as has been noted with other biological end points. The estimated dose-averaged LET of the ^{40}Ar beam is 650 keV/μm, if this were so it would suggest that the RBE did not decrease at the very high LETs (>100-200 keV/μm) as has been reported for cell killing and mutagenesis. However it is known that the ^{40}Ar beam reaching the cells of the Harderian gland is fragmented and the LET values of the fragments have not been determined. In the case of the ^{56}Fe the plateau region of the beam was used. The LET was estimated to be 190 keV/μm and presumably fragmentation of the beam was at a minimum.

The RBE values for the tumorigenic effect of the various ions are shown in Table 2. It can be seen that the RBEs range from a value of 5 for ^{4}H to 27 for ^{40}Ar and ^{56}Fe, the beams with the highest nominal LETs of the ions under study.

DISCUSSION

There has been no systematic experimental examination of the relationship of RBE to LET. The experiments reported here are an initial attempt to do just that. However, it will be necessary to use the plateau

part of the beams of ions to provide a range of LETs in order to avoid the problems produced by fragmentation of the beam by the type of filter we used to spread the Bragg peaks. Nevertheless, the results indicate an increase in effectiveness for tumor induction with increasing LET. It is of interest that the RBE for ^{56}Fe ^{40}Ar and fission neutrons appear to be similar. These results suggest the possibility that the RBE reaches a maximum, as has been found for other biological effects (18,20,22,23). There is no evidence from our results that the RBEs decrease with exposure to the beams with the highest LETs. It remains to be determined whether such a decrease occurs with the plateau part of beams with very high LETs (>500 keV/μm) as is the case for other end points.

The information about induction of cancer by heavy ions is unfortunately scanty. The efects of single and split doses of ^{40}Ar on the induction of skin tumors have been reported (26). The response to single doses was said to be approximately linear and there was no evidence of repair in the case of the split doses.

The interpretation of the tumorigenic effects of heavy ions will improve as more information is acquired about fluences and track structures. The plateaus of the curves seen in Fig. 3 are of interest. The cells with a potential for tumor production must decrease with increasing dose and inactivation and yet the tumor prevalences remain at about the same level over a wide range of doses. From data not presented here the curves for all the radiation qualities appear to plateau at a similar prevalence level and at doses that differ by factors that are not constant with the RBEs for cell killing. More information is needed before we can interpret the dose-response curves.

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Table 1

The experimental design

Radiation	Energy (MeV/amu)	S.O.B.P.*	Dose (rad) 5	10	15	20	40	80	160	320	700
^{60}Cobalt							292	280	180	181	90
Helium	910	Distal				211	201	250	257	210	40
Carbon	400	Distal	143	100	-	-	194	112	109	63	-
Neon	425	Distal	226	190	97	195	121	144	111	65	-
Neon	670	Plat.	80	50	-	44	45	44	-	-	-
Argon	570	Distal	168	120	80	126	102	154	151	-	-
Iron	600	Plat.	217	193	113	124	74	-	95	-	-

*Spread out Bragg peak.

Unirradiated implant controls: 200

Table 2

RBE Values for Heavy Ions

Radiation		RBE*
Helium-4	228 MeV/amu	5
Carbon-14	400 MeV/amu	12
Neon-20	425 MeV/amu	18
Argon-40	570 MeV/amu	27
Iron-56	600 MeV/amu	27

*RBE has been determined as follows:

$$RBE = \frac{\alpha_H}{\alpha_\gamma}$$

Where H is the initial slope of the dose response curve of the individual heavy ion beams, and α_γ is the slope for the ^{60}Co gamma ray response.

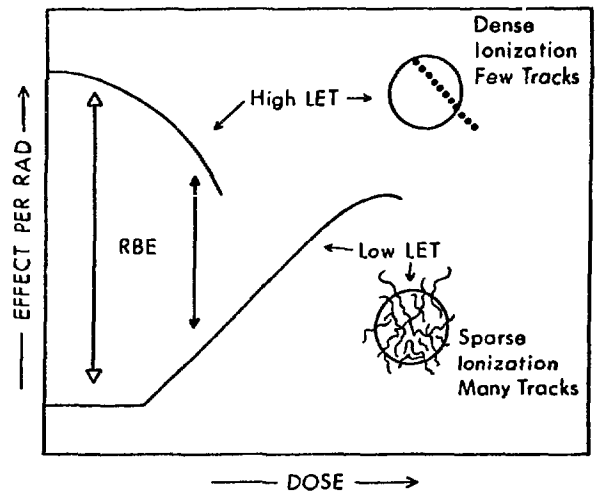
Figure Legends

FIG. 1. Schematic diagram to illustrate how RBE while LET dependent varies with the dose of both the high-LET radiation and the reference low-LET radiation. The dose levels at which the effect per rad becomes constant varies with the type of radiation and the tissue-tumor system under study.

FIG. 2. Depth-dose distributions for the ion beams. The position of the mice in the spread Bragg peaks are indicated by the arrows. In the case of ^{56}Fe the mice exposed to the plateau part of the beam.

FIG. 3. Prevalence of Harderian gland tumors as a function of dose of heavy ions as indicated.

FIG. 4. Incidence of Harderian gland tumors as a function of dose. The data for ^{56}Fe and ^{40}Ar are the same as shown in Fig. 4. The tumor rates after exposure to JANUS fission neutrons are adjusted cumulative incidences.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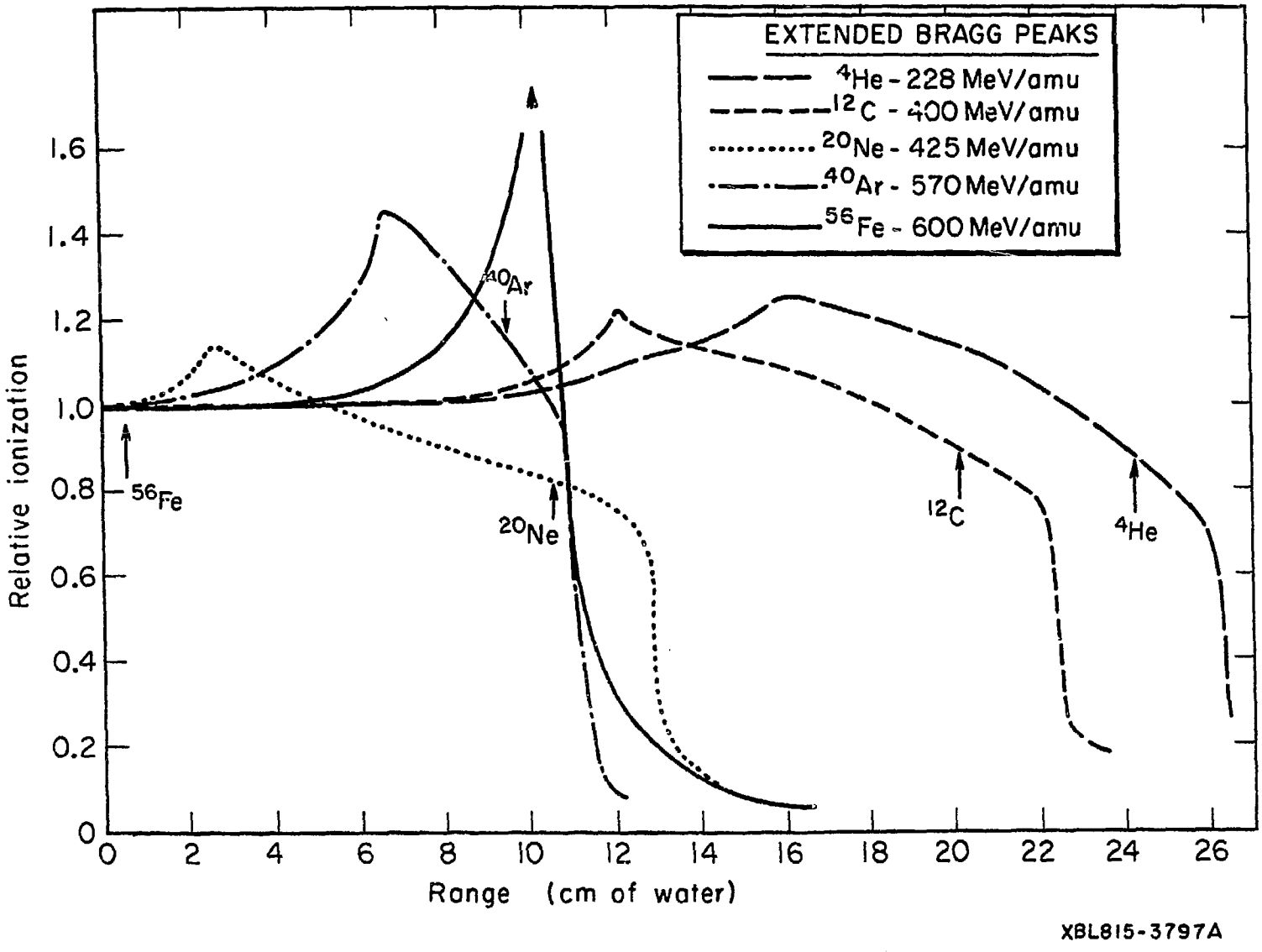

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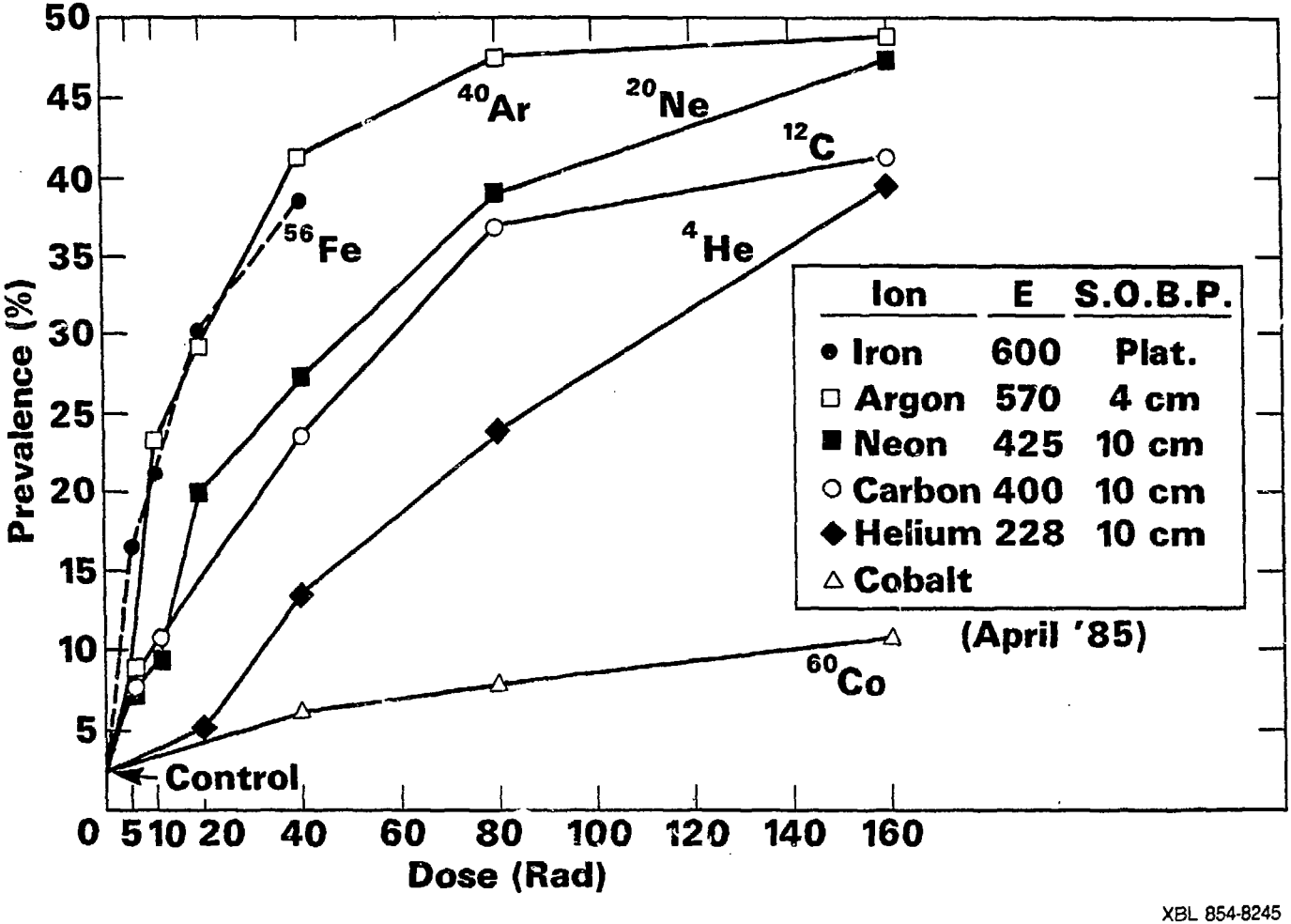
Harderian Gland Tumors



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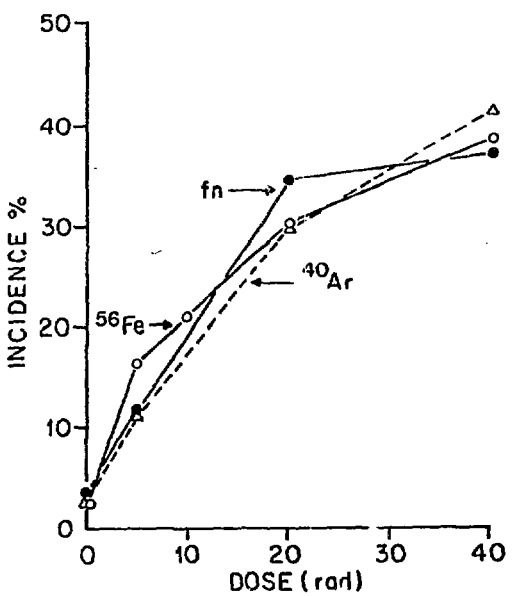
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