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BIOLOGICAL EFFECTIVENESS OF 14 MEV NEUTRONS: SPLEEN AND THYMUS WEIGHT LOSS IN MICE AS THE BIOLOGICAL INDICATOR

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HEALTH AND BIOLOGY

ABSTRACT

Atrophy of the spleens and thymuses of CF1 female mice has been used as a test system for the determination of the biological effectiveness of 14 Mev neutrons compared with that of 250 KVP X rays. The results showed that a dosage of 1×10^8 n/cm^2 was equivalent to 1 r of X rays. On the basis of rep calculated from theoretical single collision curves, the data showed that the RBE of 14 Mev neutrons was approximately 1.6. Therefore, the RBE of the 7 Mev recoil protons must be between 1 and 2 for the biological indicator used in this study. (auth)

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1. Introduction

High energy neutron sources have been used for biological studies for a number of years. Data are available from such varied sources as cyclotrons, linear accelerators, polonium- and radium-beryllium sources, and nuclear reactors. Accelerators can be used as sources of essentially monoergic neutrons. Unfortunately the beam currents and neutron fluxes available preclude, in most instances, large animal investigations and/or the use of statistically significant numbers of animals.

The Los Alamos Cockcroft-Walton accelerator is an excellent source of high energy neutrons. The use of a tritium target as described by Graves and others[1] yields relatively high fluxes of neutrons with energies in the neighborhood of 14 Mev. Measurement of the source strength in neutrons per second is very accurate and the arrangement of the apparatus minimizes the presence of contaminant radiations in the region of the source.

Several biological experiments have been carried out with this source. Cogan et al.[2] found that approximately 1×10^8 14 Mev n/cm^2 was equal to 1 r of X rays in producing epilation in rabbits, and that approximately 1.6×10^7 n/cm^2 equaled 1 r in producing lens opacities in the same species. In 1949 Carter[3] performed a preliminary study in which he found that 1.2 to 1.6×10^8 n/cm^2 was equivalent to 1 r when atrophy of the spleen and thymus of mice was used as the biological indicator of effect. It was not feasible to use large numbers of animals in either of the above experiments. At the time of Carter's work the source strength was low and only a few animals were exposed at a single point. In Cogan's experiments the size of the rabbit precluded the use of large numbers of animals.

An initial study in which lethality was the biological indicator was done by the present investigators. The results indicated that a flux near 1.3×10^8 n/cm^2 was equivalent to 1 r.[4] However, apparent inhomogeneities in the mouse population, difficulties in exposure technique, and resultant wide error bands partially negated the result.

The present study was undertaken to repeat Carter's attempt to determine the biological effectiveness of 14 Mev neutrons as compared to that of X rays. Weight loss of the spleen and thymus of mice was used as the biological indicator. Better exposure methods were devised, certain supplementary measures were incorporated, more animals were used and the effects at several dosage levels of neutrons were determined.

2. Methods

2.1 Radiation Exposure Methods

The X-ray exposures were carried out in a manner similar to that reported for other experiments.[5,6] In the present study the X radiation was delivered at the rate of 18 r/min.

The target-specimen distance was 95 cm and the source was a Picker Industrial 260 KVP machine operating at 15 ma and 250 KVP with 0.3 mm Cu added filtration.

The exposures to the 14 Mev neutrons were made with the Los Alamos Cockcroft-Walton accelerator. The 14 Mev neutrons were produced in a tritium target bombarded by deuterons. The neutrons are emitted from the target in all directions. Alpha particles are emitted concurrently with the neutrons in a 1:1 ratio. The neutron source strength was determined by counting the target alpha particles. The target source strength was found to vary with the "age" of the target. Thus, the source strength gradually decreased with time. For this experiment, the flux at the exposure distance of 12 cm from the center of the target varied from 1.3×10^7 to 1.7×10^7 n/cm^2/sec.

The animals were exposed 30 at a time in a hemispherical lucite cage so arranged that the center of the group of animals was 12 cm from the target. The exposure arrangement is shown in Fig. 1. Cooled air was blown through the cage during exposure to insure adequate ventilation and to prevent over-heating of the mice. Since the source strength was known, it was simple to calculate the neutron flux to the animals based on distance to the center of the exposure cage. The total flux given to each group was determined directly from the alpha counts determined during exposure.

To determine the variation of flux over the surface of the cage, copper foils were activated and relative activities determined. Copper foils were also used to determine the decrease in flux from proximal to distal surfaces of the exposure cage.

2.2 Biological Methods

The use of the spleen and thymus as a biological indicator of radiation effect was first described by Carter et al.[7] The mice used were CF1 females procured from a commercial source at 5 to 7 weeks of age. The population was allowed to stabilize in the local laboratory environment for approximately 2 weeks prior to exposure. The day preceding exposure, the animals were randomized and those with weights of less than 21 gm or more than 25 gm were eliminated. The mice were divided into two large groups for X-ray and neutron exposures, respectively. After exposure the animals were kept under normal laboratory conditions for 5 days and sacrificed 120 ± 3 hours post-exposure. The splenic wet weights were determined immediately after sacrifice. The thymuses were fixed in 10% formalin for 24 hours to facilitate removal of excess fat and connective tissue. At that time the tissues were blotted dry and weighed to the nearest milligram.

3. Experimental Results

The results of the copper foil studies are shown in Table 1 and in Fig. 2. The relative flux and neutron energy values are also shown in Table 1. The neutron energy had only a narrow spread from 13.7 to 14.9 Mev. The fluxes found over the limits of the exposure area varied over a range of 5%. The 25% variation in copper foil activity shown by the uncorrected data in Fig. 2 was due to the rapidly changing cross section with small variation in energy.

The biological results are shown in Table 2. The resultant curves from these data are given in Figs. 3 and 4. The regression lines shown in Figs. 3 and 4 were established by least squares analysis of the data in Table 2, using time of exposure as the common independent variable. To have a common independent variable it was necessary to assume an average rate of delivery of neutron irradiation because of decrease in source strength with time. An average flux to the animals of 1.5×10^7 $n/cm^2/sec$ during the exposure was calculated. Also, it is necessary to assume that the biological effectiveness does not vary over the rather narrow change in dose rate. Gamma ray experiments support the validity of this assumption.[8] The X-ray exposures were made at a dose rate of 0.303 r/sec to be more commensurate with the predicted neutron exposure rate. The formulae for the regression lines in Figs. 3 and 4 and the relative potencies of the two radiations for the production of weight loss in both spleen and thymus were evaluated in the following manner.

The data were fitted by least squares analysis to the general regression equation $Y = (\bar{y} - b\bar{x}) + bx$. In this equation x is the log of the time of exposure and $\bar{x}$ is the mean, Y is the percentage of organ weight loss and $\bar{y}$ is the mean, and b is the regression coefficient or slope in units of the dependent variable. The regression lines developed are represented by the following formulae.

For the weight loss of the spleen following

X-ray exposure $Y_x = -87.11 + 49.02\, X_x$

14 Mev neutron exposure $Y_n = -102.65 + 49.02\, X_n$.

For the weight loss of the thymus following

X-ray exposure $Y_x = -170.64 + 77.38\, X_x$

14 Mev neutron exposure $Y_n = -191.65 + 77.38\, X_n$.

An analysis of variance indicated that the slopes of the two sets of regression lines were not significantly different.

The relative potency of 250 KVP X rays and 14 Mev neutrons in terms of the effect on the two biological test systems may be determined by the equation $\text{Log } P_{xn} = \dfrac{\bar{y}_x - \bar{y}_n - b(\bar{x}_x - \bar{x}_n)}{b}$ where P_{xn} is the relative potency of X radiation compared to neutron irradiation. The values found were:

Splenic response $P_{xn} = 2.075 \pm 0.238$

Thymic response $P_{xn} = 1.869 \pm 0.121$.

Since 1 sec of exposure was equivalent to 0.303 r of X rays or 1.5×10^7 n/cm^2, the number of 14 Mev neutrons equivalent in biological effect to 1 r of 250 KVP X rays may be calculated from the expressions for relative potency given above.

When splenic weight loss is used as the biological indicator, $(1.03 \pm 0.12) \times 10^8$ n/cm^2 = 1 r, and when thymic weight loss is used, $(0.93 \pm 0.06) \times 10^8$ n/cm^2 = 1 r. The average value for the two biological systems is 0.96×10^8 n/cm^2 = 1 r of 250 KVP X rays with an error of less than ± 20%.

4. Discussion of Results

In any determination of relative biological effectiveness of two radiations it is necessary to determine in ergs or rep, the energy delivered to tissue by the radiations. In the present study it was not possible to make physical determinations of neutron dose in ergs or rep and, as in previous work with 250 KVP X rays, equivalence of r, rem, and rep is assumed. Since no instruments were available for the determination of neutron dose in energy units, it is necessary to use theoretical values for the energy imparted to tissue. Using cross-section data from AECU-2040, Hurst[9] calculated the energy deposited per gram of tissue on a first collision basis. The average tissue composition was assumed to be 10% hydrogen, 73% oxygen, 12% carbon, and 4% nitrogen by weight. The theoretical curve of $rep/n/cm^2$ versus energy in Mev is plotted in Fig. 5. This curve indicates that the tissue dose at a neutron energy of 14 Mev is 6.5×10^{-9} $rep/n/cm^2$. Approximately 85% of the total dose is due to recoil hydrogen nuclei or protons of 7 Mev energy. The remainder of the dose is due to the recoil of oxygen, carbon, and nitrogen nuclei with energies of 1 to 2 Mev.

Combining the weighted values found from the spleen and thymus measurements $\left((0.96 \pm 0.09) \times 10^8 \ n/cm^2 \text{ equivalent to } 1 \text{ r}\right)$, then 0.624 ± 0.058 rep of energy is equivalent in biological effectiveness to 1 r of 250 KVP X radiation. If equivalence of roentgen and rem is assumed, the RBE of 14 Mev neutrons becomes 1.60 ± 0.15, using spleen and thymus weight loss as the biological indicator of radiation effect. If, in considering the contributions of each recoil nucleus to dose, one assumes instead a ridiculously low RBE of 0.1 for the protons, then the maximum RBE of the heavier recoils becomes 10. If on the other hand the proton is assumed to have an RBE of 1.0, then the heavier nuclei have an RBE of only 5. Assuming that the heavier recoil nuclei contribute nothing to the dose, the proton RBE is still less than 2.0. Thus, the experimental results indicate that the RBE of 7 Mev protons calculated on a single collision basis must be between 1 and 2 for this particular biological response.

5. Summary

The relative effectiveness of 14 Mev neutrons produced by deuteron bombardment of a tritium target in the Los Alamos Cockcroft-Walton has been compared with that produced by 250 KVP X radiation, with the weight loss of the spleens and thymuses of CF1 female mice being used as the indicator. The results show that 1×10^8 n/cm^2 is equivalent to 1 r of X rays and that the RBE of the neutrons is 1.6 ± 0.15 if theoretical calculations of rep for a single collision process are used. From these results the RBE of 7 Mev protons must be between 1 and 2, and the RBE of carbon, nitrogen, and oxygen recoil nuclei must be between zero and 5.

6. References

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8. P. S. Harris, unpublished work, Los Alamos Scientific Laboratory (1952).
9. G. S. Hurst, personal communication (1953).

TABLE 1

STUDIES OF THE NEUTRON SOURCE WITH COPPER FOILS IN

THE EXPOSURE REGION

Angle to Incident Beam (degrees)	Relative Cu Foil Activity. Uncorrected for Energy Change	Neutron Energy (Mev)	Relative Neutron Flux Corrected
0	1.00	14.89	1.00
30	0.98	14.77	0.99
60	0.89	14.49	0.97
90	0.78	14.10	0.95
120	0.65	13.72	0.92

TABLE 2

SPLEEN AND THYMUS WEIGHT LOSS FROM 14 MEV NEUTRON AND X RADIATION

Dose*	Time of Exposure (sec)	Spleen Wt (mg)	Spleen Wt Lost (%)	Thymus Wt (mg)	Thymus Wt Lost (%)
Control		103.5	--	91.0	--
200 r X radiation	660	51.4	50.3	49.2	46.1
280 r X radiation	924	43.7	57.8	31.4	65.5
400 r X radiation	1320	33.6	67.5	28.5	68.7
560 r X radiation	1848	26.8	74.1	16.0	82.4
790 r X radiation	2604	21.8	78.9	8.8	90.3
1.30×10^{10} n/cm^2	867	53.0	48.8	60.4	33.6
1.82×10^{10} n/cm^2	1213	60.2	41.9	48.3	46.9
2.60×10^{10} n/cm^2	1733	50.0	51.7	39.9	56.1
3.64×10^{10} n/cm^2	2427	37.2	64.1	25.9	72.6
5.20×10^{10} n/cm^2	3467	28.7	72.3	12.3	86.5
7.28×10^{10} n/cm^2	4853	21.3	79.4	7.3	92.0
10.80×10^{10} n/cm^2	7333	All dead prior to sacrifice			

*X radiation delivered at a dose rate of 0.303 r/sec.

Neutrons delivered at an average dose rate of 1.5×10^7 n/cm^2/sec.

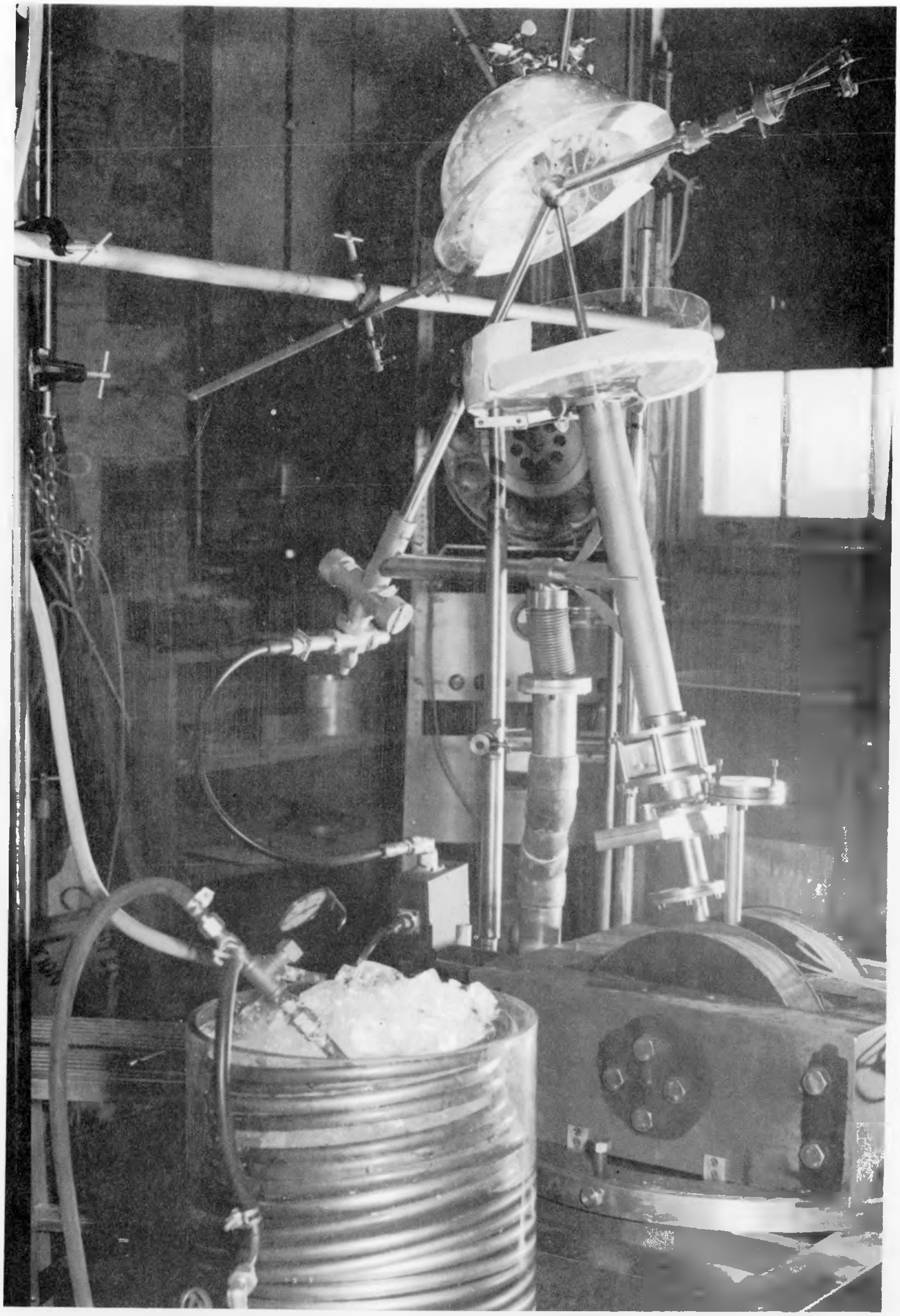

Fig. 1 Exposure arrangement for 14 Mev neutron studies.

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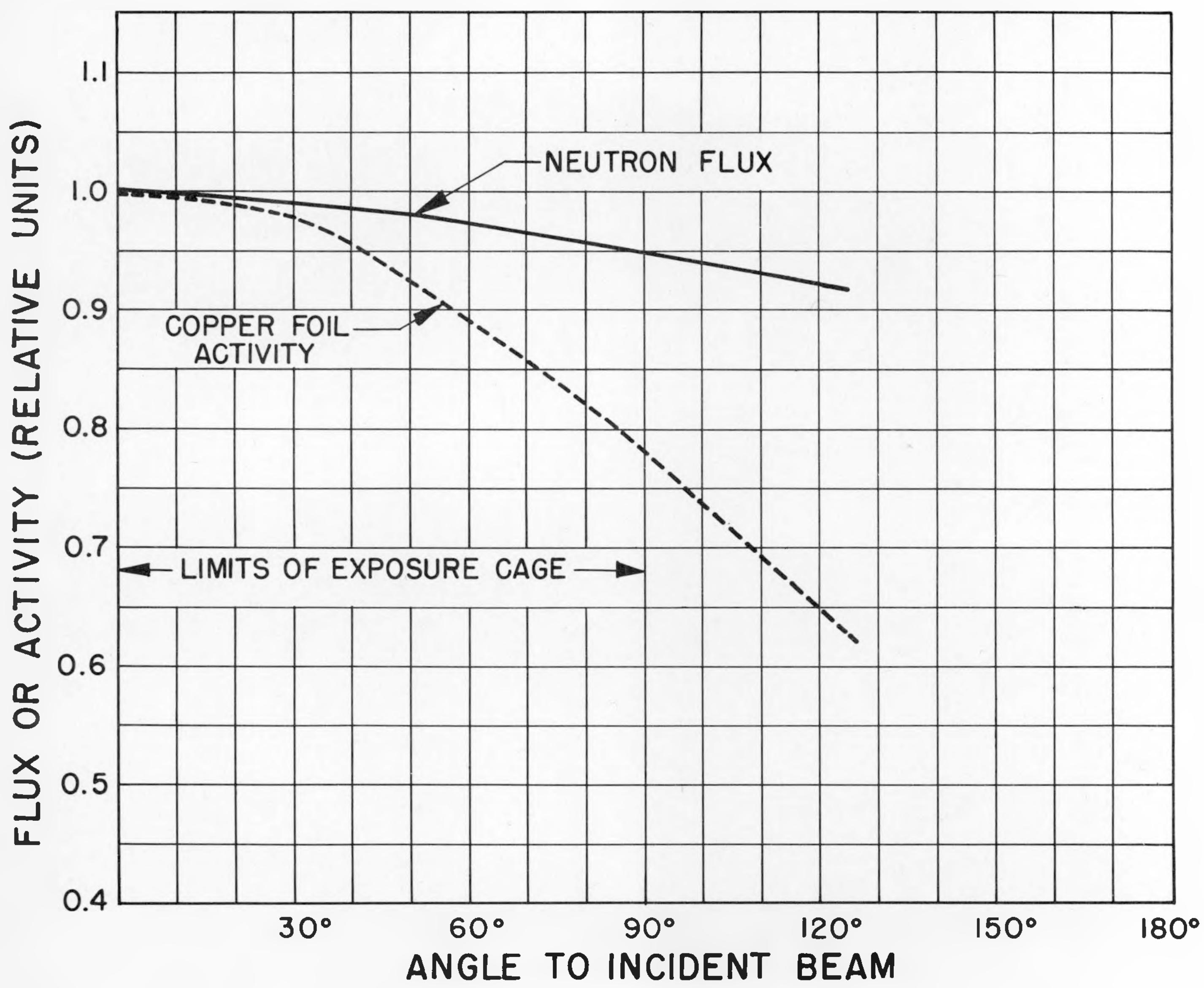

Fig. 2 Variation of copper foil activity (uncorrected) and neutron flux with angle to the incident beam.

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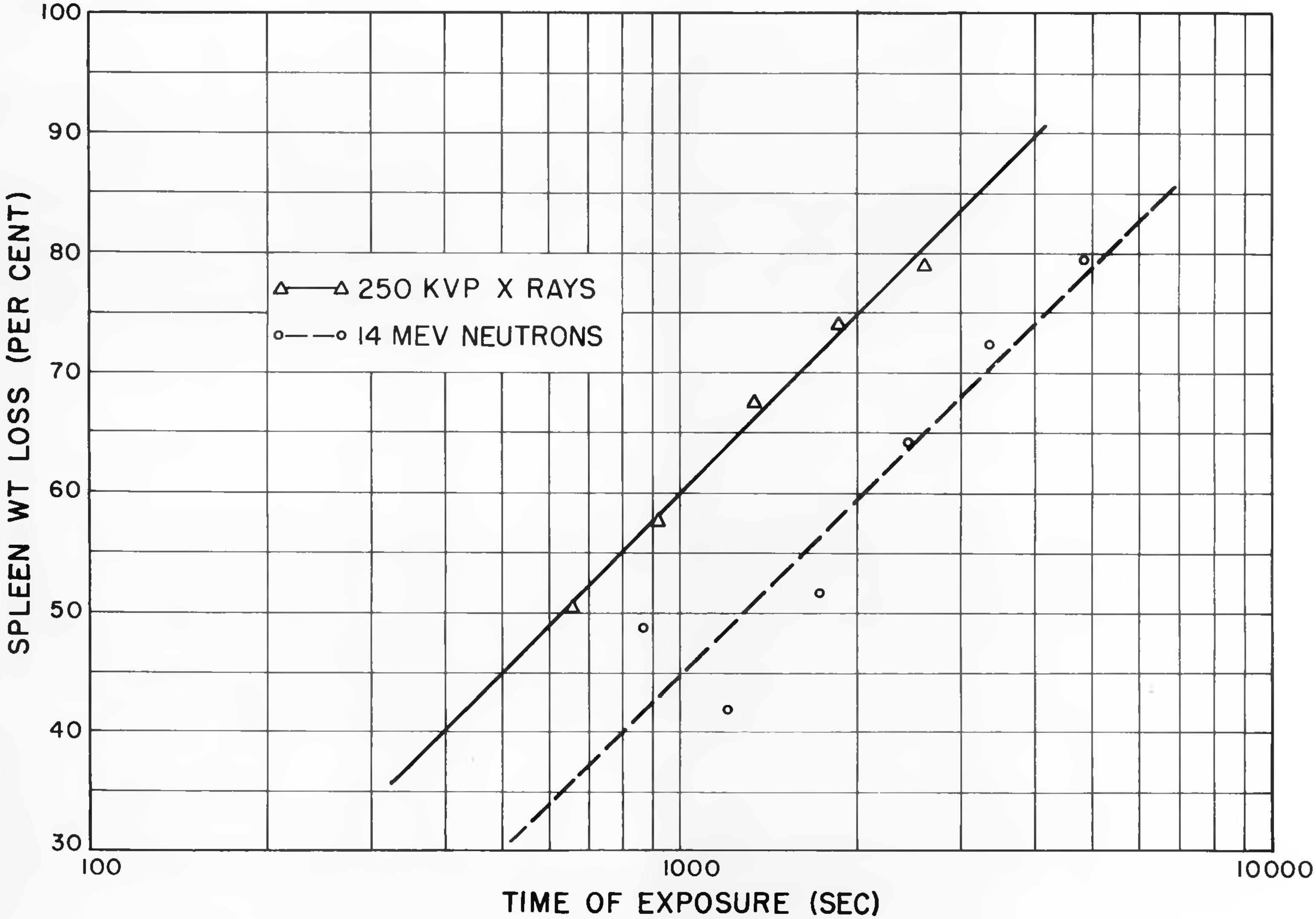

Fig. 3 Decrease in spleen weight following exposure to X rays and to 14 Mev neutrons.

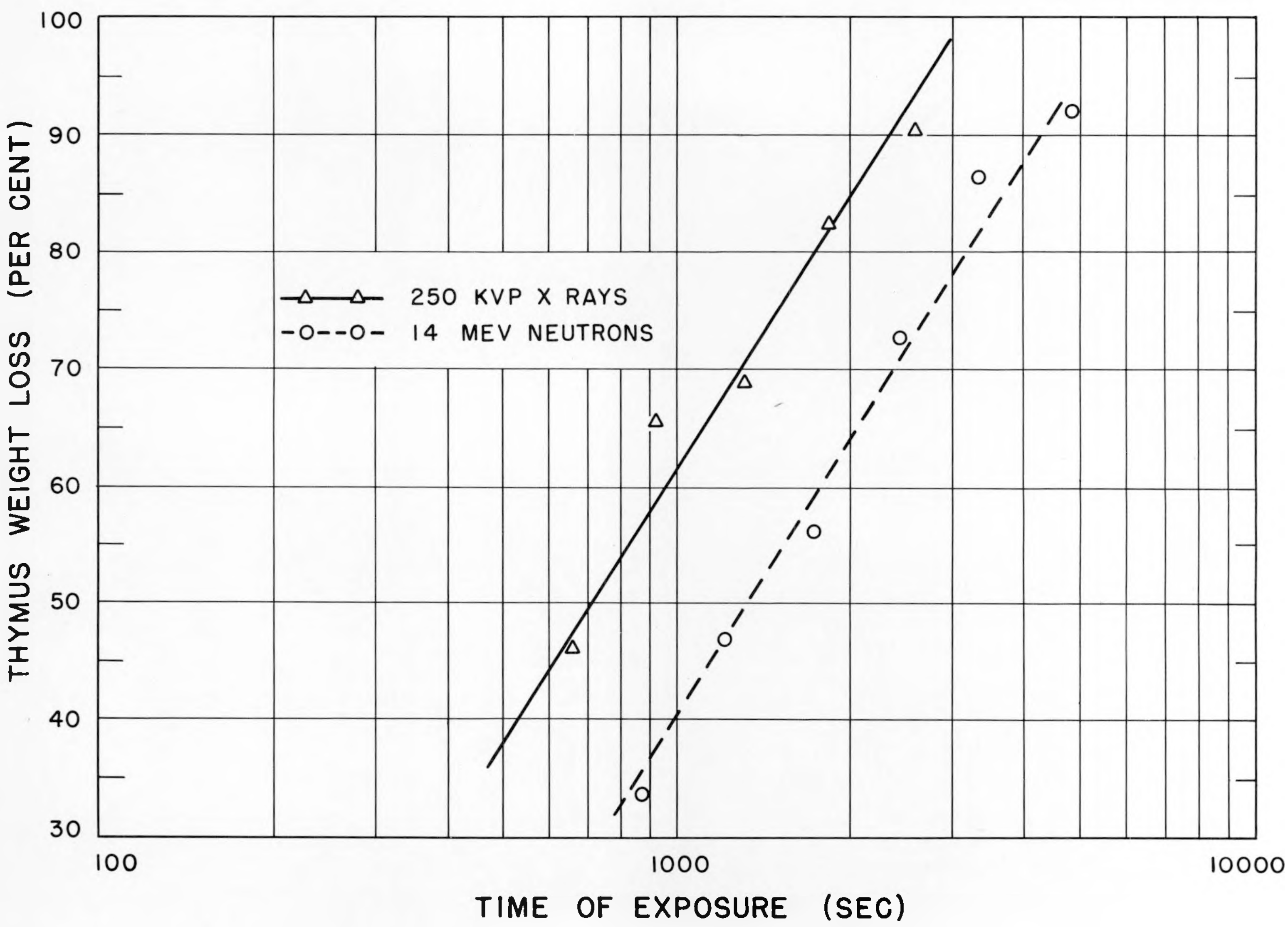

Fig. 4 Decrease in thymus weight following exposure to X rays and to 14 Mev neutrons.

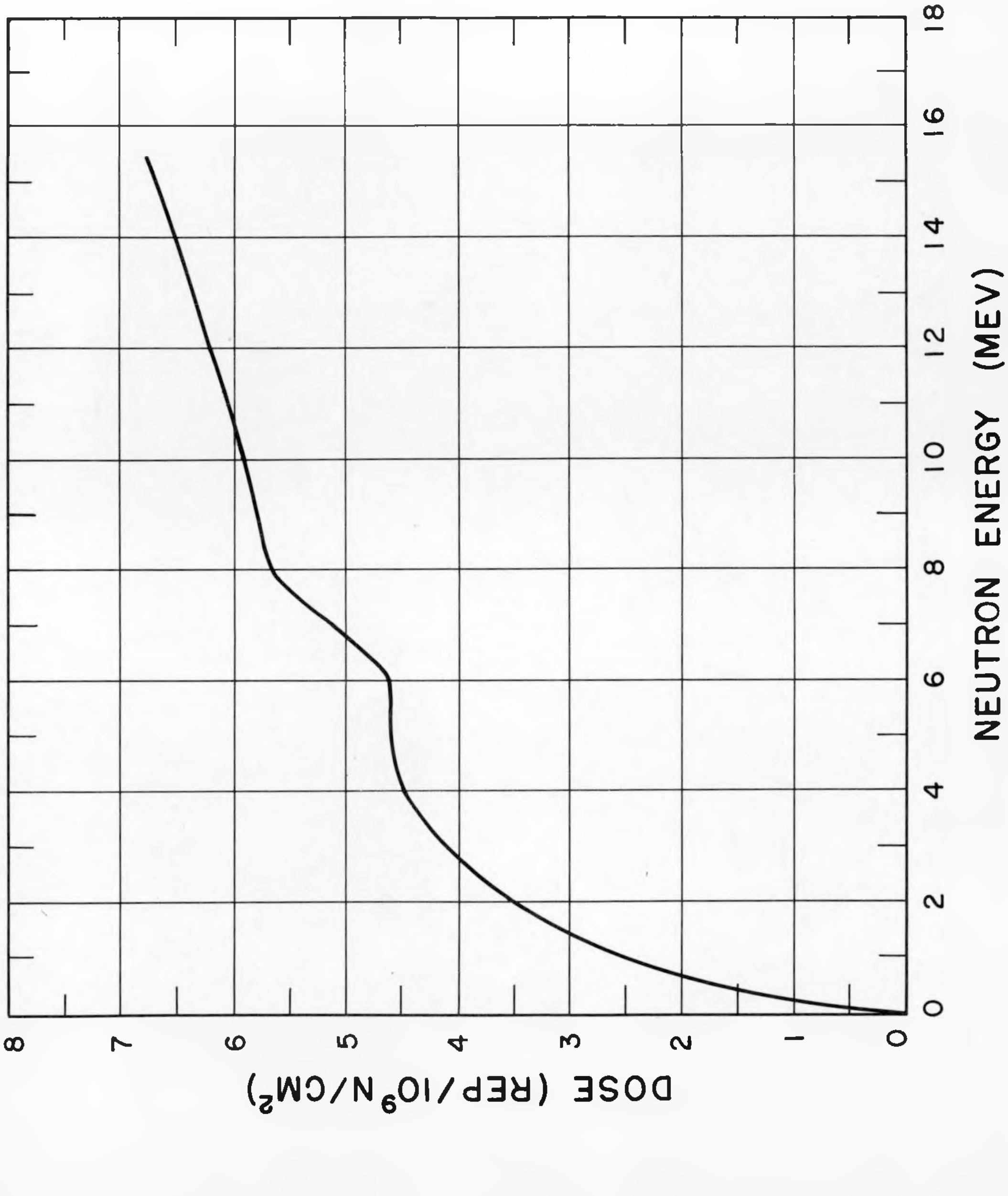

Fig. 5 Theoretical variation of dose in rep/n/cm^2 in tissue with changing fast neutron energy.