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THE BIOLOGICAL EFFECTIVENESS OF THERMAL NEUTRONS DETERMINED BY THE DECREASE IN WEIGHT OF THE SPLEEN AND THYMUS OF THE MOUSE

Work done by:

Payne S. Harris[a]
James T. Brennan[b]
Robert Emerson Carter[c]

Report written by:

Payne S. Harris
James T. Brennan

[a] Major, MC, USA, Assigned 8452nd SWG, Sandia Base with Station at Los Alamos.

[b] Lt. Col., MC, USA. Present address: AMSGS, AMC, Washington, D. C.

[c] Group P-2, LASL.

HEALTH AND BIOLOGY

ABSTRACT

Female CF-1 mice were exposed to various radiation sources, including a 250 KVP X-ray machine and a homogeneous reactor. Regression curves of radiation response versus exposure were determined using the weight loss of the spleen and thymus as a biological indicator of radiation effect.

These results indicated that the gamma radiation produced in the homogeneous reactor had a biological effectiveness of 0.6 to 0.8 when compared to the effect of the 250 KVP X-ray spectrum.

The biological effectiveness of thermal neutrons determined by this method indicated that $1.25 \pm 0.25 \times 10^{10}$ thermal neutrons/cm^2 were equivalent to 1 r of the X radiation.

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THE BIOLOGICAL EFFECTIVENESS OF THERMAL NEUTRONS DETERMINED BY THE DECREASE IN WEIGHT OF THE SPLEEN AND THYMUS OF THE MOUSE

1. INTRODUCTION

The biological effectiveness of thermal neutrons has recently been determined by lethality observations in mice.[1] The same determination using another biological indicator and similar radiations would be useful as an extension and check of these results.

The data and conclusions given below were obtained by using the splenic and thymic weight loss method as a biological indicator of radiation effect.[2] The physical facilities utilized in this experiment were the same as those used in the lethality studies.[1]

2. SPLENIC AND THYMIC WEIGHT LOSS IN MICE AS A BIOLOGICAL INDICATOR

Atrophy of the spleen and thymus in experimental animals exposed to ionizing radiation has been noted by various investigators since 1904. A systematic quantitative investigation of these weight changes has been carried out at this laboratory to determine the feasibility of the response as a laboratory and a field test procedure. More complete discussion of the method can be found elsewhere.[3,4]

In essence the results of these studies indicate that weight loss of either the spleen or thymus of the mouse is exponentially related to the quantity of ionizing radiation to which the animal has been exposed. The response of either organ may adequately be described by the equation:

$$\log Y = a + bx$$

where

Y = the dose (or some functionally related quantity, i.e., time of exposure) of ionizing radiation given to the animal,

x = per cent weight loss of the spleen or thymus of the experimental animal when compared to unirradiated controls,

a = the intercept constant of the formula determined from least squares computation of the regression line,

b = the regression coefficient or slope of the regression line.

By comparison of regression lines for two different radiations the biological effectiveness of one in terms of the other can be determined. In these experiments, all effectiveness factors have been developed in terms of the response of the spleen and thymus to 250 KVP X radiation.

3. EXPERIMENTAL METHODS

3.1 Biological Methods

CF-1 female mice were used throughout the experiment. The animals were obtained from a commercial source at five to seven weeks of age and allowed to stabilize at the Los Alamos altitude for two weeks prior to exposure. One day previous to exposure the mean weight of the group was determined and all animals with weights outside two standard deviations from the mean were excluded. Animals within the weight range were then randomized into groups of thirty for control and exposure purposes.

The animals were maintained for five days after exposure with food and water in constant supply. At 120 $\pm$ 3 hours post-exposure all groups were sacrificed. Killing was accomplished with ether. The spleens and thymuses were removed, and the wet weight of each spleen was determined immediately. Each thymus was placed in 10 per cent formalin for twenty-four hours to facilitate the removal of excess fat and connective tissue. Following this fixation and removal of foreign matter, each thymus was blotted dry and weighed.

The mean per cent splenic and thymic weight loss for each group of thirty animals was determined by comparison with the mean spleen and thymus weight of a group of non-irradiated controls. Weights falling outside the limits of the Chauvenet Criterion (i.e., 2.4 standard deviations from the mean for a thirty sample group) were discarded and the new mean calculated. Application of this test resulted in the rejection of less than one per cent of the samples. The regression curves needed for effectiveness determinations were found from these data by the method of least squares.

3.2 Exposure Methods

Three exposure systems were used. The north thermal column of the Los Alamos homogeneous reactor (a full discussion of this reactor may be found in other reports[1,5]) was used for the neutron and thermal column gamma determinations. A 250 KVP X-ray machine was used to establish the baseline response curves.

The X-ray exposure conditions are shown in Table 1. The animal exposure assembly is shown in Fig. 1. Six separate experiments on different groups of mice with day to day changes in intensity account for the dose rate variations shown in the table.

The exposures to the mixed thermal column radiation were made at a position in the north thermal column of the homogeneous reactor indicated in Fig. 2. In this group of experiments the center of the exposure cage was four inches closer to the radiating sphere than in the lethality experiments,[1] and the diameter of the graphite cavity was six inches instead of eight inches. No cylindrical bismuth shield was used around the exposure cage. The pertinent exposure conditions are shown in Table 1. The poor geometry lithium absorption curve is shown in Fig. 3. The thermal column gamma dose rate shown in the table was determined by a method similar to that used by Brennan et al.[1]

The exposures to thermal column gamma only were made at the same position as the lethality exposures.[1] The exposure conditions are shown in Table 1 and Fig. 2.

4. EXPERIMENTAL RESULTS

4.1 X-Ray Exposures

Six different experiments were made at different times. The dosage range varied from one set of exposures to another. The results of all experiments were combined to furnish the baseline regression curves. The combined data are shown in Table 2. Each value for per cent weight loss is the mean of all observations at a particular dosage and was weighted for the number of observations. The regression lines for splenic and thymic weight loss are shown in Figs. 4 and 5 respectively. The 93 per cent confidence limits are shown by the error bands.

For the spleen the regression formula is:

$$\log Y = 1.54926 + .017828\ x \quad (50 \leq Y \leq 1000)$$

and for the thymus

$$\log Y = 1.56716 + .015567\ x \quad (50 \leq Y \leq 1000)$$

where

Y = dose in roentgens,

x = per cent organ weight lost.

4.2 Thermal Column Gamma Exposures

The results of the exposures to the thermal column gamma radiation only are shown in Table 3. The regression lines developed from the data are shown in Figs. 6 and 7.

The data for the spleen is given by the formula:

$$\log Y = 1.75813 + .015668\ x \quad (100 \leq Y \leq 1000)$$

and for the thymus by the formula,

$$\log Y = 1.86987 + .014116\ x \quad (100 \leq Y \leq 1000)$$

where

Y = dose in roentgens (for a 4 Mev gamma ray), and

x = per cent organ weight lost.

4.3 Thermal Column Mixed Radiation Exposures

The data accumulated from exposures to the mixed thermal column radiation are shown in Table 4. The regression lines are shown in Figs. 8 and 9. In these exposures the $\underline{Y}$ values are in units of time of exposure in seconds.

The regression line equations are:

for the spleen,

$$\log Y = 1.58559 + .014614\ x \quad (94 \leq Y \leq 658)$$

for the thymus,

$$\log Y = 1.63280 + .012287\ x \quad (94 \leq Y \leq 658)$$

4.4 Statistics

The regression line constants and statistical parameters used in the final interpretation of the data are shown in Table 5. Inter-comparisons of the various separate X-ray exposure experiments denote a high degree of homogeneity and internal consistency. The variances shown in the table were used to determine the fiducial limits of the regression curves at the 93 per cent confidence levels.* These limits appear on the various figures. The variances in the slopes (or regression coefficients) were used in significance testing between various experiments. Results indicate that the probability of chance causing slope variations of the magnitude shown by the data varies from 10 to 50 per cent depending on the range of data tested and the organ being measured.

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* At the 93 per cent confidence level, the fiducial limits may be determined from the formula,

$$\pm 2\sqrt{V(a) + V(b)\ (x - \bar{x})^2},$$

where $\bar{x} = \sum x/n$.

5. INTERPRETATION OF RESULTS

5.1 Comparison of the Effect of Thermal Column Gamma Radiation and 250 KVP X Radiation

To determine finally the biological effectiveness of thermal neutrons, it was necessary to subtract from the mixed thermal column radiation response curves those portions of the responses due to factors other than neutrons. Of most importance, of course, was the pile gamma effect.

One method for evaluating the gamma effect was to determine the gamma to X-ray effectiveness ratio and subtract it from the total pile response curves in terms of the baseline X-ray radiation.

As a general solution for effectiveness determinations by the spleen-thymus method, the following system may be used:

Consider two radiations, m and n, whose regression lines for either spleen or thymus response are represented by the formulae:

$$\log Y_m = a_m + b_m x_m \qquad (1)$$

and

$$\log Y_n = a_n + b_n x_n \qquad (2)$$

Y_m and Y_n are physical dose measurements or measurements (i.e., time of exposure) linearly proportional to physical dose.

E_m and E_n may be defined as biological effectiveness factors such that:

$$Y_m E_m = \text{rem of radiation m}$$

and

$$Y_n E_n = \text{rem of radiation n}$$

Subtracting (2) from (1) and substituting

$$\log E_n - \log E_m = \log(rem_n) - \log(rem_m) + a_m - a_n + b_m x_m - b_n x_n.$$

If m is considered to be the baseline radiation $E_m = 1$, and at a point where $rem_m = rem_n$, $x_m = x_n$ and

$$E_n = \frac{\text{antilog } a_m}{\text{antilog } a_n} \; 10^{(b_m - b_n)x}$$

If the regression lines are parallel, $b_m = b_n$, and

$$E_n = \frac{\text{antilog } a_m}{\text{antilog } a_n}$$

Thus, the biological effectiveness of one radiation in comparison to a baseline radiation is just the inverse ratio of the antilogs of the intercept constants.

A comparison of the thermal column gamma and X-ray curves from these experiments shows that in no case is $b_m = b_n$, and in no case are the regression lines absolutely parallel. The factors that may contribute to this slope variation are discussed in a later section. However, values for effectiveness may be determined in spite of this variation.

One method for the determination of E_n may be called the area method. With this method a computation is made of the ratio of the areas under the two curves over the unextrapolated range.

In this case the respective areas are:

For radiation m:

$$Y_m \, dx_m = \int_{x_{m_1}}^{x_{m_2}} 10^{a_m + b_m x_m} \, dx_m$$

For radiation n:

$$Y_n \, dx_n = \int_{x_{n_1}}^{x_{n_2}} 10^{a_n + b_n x_n} \, dx_n$$

Since $Y_m \sim \frac{1}{E_m}$, $E_m = 1$ and $Y_n \sim \frac{1}{E_n}$

$$E_n = \frac{\int_{x_{m_1}}^{x_{m_2}} 10^{a_m + b_m x_m} \, dx_m}{\int_{x_{n_1}}^{x_{n_2}} 10^{a_n + b_n x_n} \, dx_n}$$

A second type of determination that can be used could be called a point analysis. In this case the formula shown previously is used:

$$E_n = \frac{\text{antilog } a_m}{\text{antilog } a_n} \; 10^{(b_m - b_n)x}$$

The value of x is chosen for a point at which the standard errors of the regression lines approach minima. This is, of course, a point at which x approaches $\bar{x}$ (the mean per cent weight loss value for the curve).

Values for the biological effectiveness of the thermal column gamma compared to X ray determined by both methods for both organs are shown in Table 6. A similar value determined by the lethality studies is also shown.

5.2 Comparison of the Effect of Mixed Radiation of the Thermal Column and 250 KVP X Radiation

For a comparison of the effect of thermal neutrons to X rays it is necessary to subtract from the curves of response to the mixed thermal column radiation all effects due to radiations other than thermal neutrons. In the previous section equivalence values were determined for thermal column gamma. The second important value needed is an effectiveness figure for intermouse gamma radiation due to the gamma rays from hydrogen capture in one animal radiating a second animal. This is proportional to the neutron flux and the number of mice. Brennan et al[1] determined in the lethality studies that a neutron flux of 1.8×10^{10} n/cm^2/sec caused an intermouse gamma dose, rem_I, equal to 0.12 rem/sec. In these experiments with a flux of 1.4×10^{10} n/cm^2/sec and an equivalent mass of mice at each exposure the intermouse gamma dose rate was $rem_I = 0.09$ rem/sec.

The dose due to the thermal column gamma, rem_γ, is equal to $r_\gamma E_\gamma$, where r_γ = roentgens of thermal column gamma. For the exposures to the total column radiation it was shown previously that the gamma dose rate at the position of exposure was 0.56 r/sec. Thus, the intensity of this fraction of dose was 0.56 E_γ rem/sec.

The regression lines for the mixed thermal column radiation are represented by:

$$\log Y_p = a_p + b_p x_p$$

$Y_p = t_p$ = exposure time in seconds.

Since dose is a linear function of exposure time, dose rate x time equals total dose and rem of neutrons + rem of thermal column gamma + rem of intermouse gamma = total dose in rem, or

$$\left(NE_n + \frac{dr_\gamma}{dt} E_\gamma + \frac{d\ rem_I}{dt}\right)t_p = \text{total rem} = \text{rem of X ray}$$

N = neutron flux in $n/cm^2/sec$

E_n = neutron effectiveness in $rem/n/cm^2$.

For these exposures the flux in the empty cage was $N_o = 1.4 \times 10^{10}$ $n/cm^2/sec$. In the lethality studies it was shown that the mice decreased the flux to each animal by 21 per cent.[1] Thus, N = 79 per cent N_o or $N = 1.11 \times 10^{10}$ $n/cm^2/sec$. Solving the above equation for E_n

$$E_n = \frac{1}{N}\left[\frac{rem_{x\ ray}}{t_p} - \frac{dr_\gamma}{dt} E_\gamma - \frac{d\ rem_I}{dt}\right]$$

$$\text{rem of X ray} = \text{r of X ray} = 10^{a_x + b_x x_x}$$

$$t_p = Y_p = 10^{a_p + b_p x_p}$$

$$\frac{1}{N} = \frac{1}{1.11} \times 10^{-10}$$

$$\frac{dr_\gamma}{dt} E_\gamma = \frac{d\ rem_\gamma}{dt} = 0.56\ E_\gamma$$

$$\frac{d\ rem_I}{dt} = 0.09$$

and

$$E_n = \frac{10^{-10}}{1.11}\left[10^{(a_x - a_p) + b_x x_x - b_p x_p} - 0.56\ E_\gamma - 0.09\right]$$

As before, solving for E_n at $x_x = x_p$ where the standard error of the regression lines approach zero determines E_n in units of $rem/n/cm^2$.

The values obtained in units of $1/E_n$ for the various biological systems are as follows:

(a) spleen weight loss 1.32×10^{10} n/cm^2/rem

(b) thymic weight loss 1.18×10^{10} n/cm^2/rem

(c) lethality studies 1.37×10^{10} n/cm^2/rem

5.3 The Reliability of the Effectiveness Values

In the considerations above, errors in dose measurement and the biological system itself contributed to the uncertainty of the final results. In general, it can be stated that the error in the flux measurement method was $\pm$ 10 per cent. The errors in ionization chamber measurements were of the order of 5 per cent.

Of special interest is the variation in slope of the different regression lines. It might be argued that the variation in slope defeats the attempt to determine a single-valued effectiveness factor for the dose range studied. Indeed, tests of significance of the combined X-ray curves indicate that such variations in slope (between X-ray curves and either pile gamma or mixed pile radiation curves) may be only 10 per cent due to chance. However, testing individual X-ray runs against the thermal column curves showed that the probability of slope differences of the order indicated can be as much as 50 per cent due to chance.

If all data are combined, the following formula can be developed:

$$E_n = \left[10^{(a_x - a_p) + (b_x - b_p)x} - 0.56\ 10^{(a_x - a_\gamma) + (b_x - b_\gamma)x} - 0.09 \right] \frac{1}{N}$$

Solving this formula for the limiting values of x (which in this case are the end points of the mixed thermal column radiation curves) gives, for both the spleen and thymus values, an error of ± 20 per cent due to slope variation over the range studied.

Also, if the ranges of the combined X-ray curves are restricted to the weight loss ranges as shown by the thermal column gamma and mixed column radiation curves, significance tests indicate very high probability of slope variation due to chance. Using such restricted ranges gives neutron effectiveness values in agreement with those shown previously.

6. SUMMARY AND CONCLUSIONS

Studies on various radiations from a homogeneous nuclear reactor have been carried out using CF-1 female mice and weight loss of the spleen or thymus as a biological indicator of radiation effect.

The results indicate that 1.25 ± 0.25 x 10^{10} thermal neutrons per cm^2 are equivalent to 1 roentgen of 250 KVP X ray. These results are in good agreement with the effectiveness value found previously in a lethality study on Swiss mice. It is apparent that the same factors, whether they be local response or total body response to radiation, are operating in both studies. The errors involved in these studies are of the same order as those in the lethality studies and in general, the method is simpler and more rapid.

7. REFERENCES

1. Brennan, J. T., Harris, P. S., Carter, Robert Emerson and Langham, W. H., LA-1408.
2. Carter, R. E., Harris, P. S., and Brennan, J. T., LA-1075.

3. Carter, R. E., et al., The Effect of X Radiation on the Spleen and Thymus of the Mouse (in manuscript).

4. Brennan, J. T. et al., Effect of Radiation in a Thermal Neutron Column on the Spleen and Thymus of the Mouse (in manuscript).

5. __________, __________, Rev. Sci. Instr. 22, 7, p 489-99 (July 1951).

TABLE 1

Radiation Exposure Conditions

X-RAY EXPOSURE

1. Unit - 250 KVP Picker Industrial Machine; Self Rectified Machlett Tube

2. Filament Current - 15 ma

3. Filtration
   Inherent - 3 mm Al
   Added - 3.8 mm Cu

4. HVL - 4.9 mm Cu

5. Target to Specimen Distance - 55 cm

6. Dose rate (free air) - 14 to 16 r/min

MIXED THERMAL COLUMN RADIATION EXPOSURE

1. Unit - Los Alamos Homogeneous Reactor; North Thermal Column

2. Power Level - 10 kw

3. Shielding - None

4. Thermal Neutron Flux - 1.4×10^6 n/cm^2/sec at 1 watt

5. Gamma dose rate - 0.56×10^{-4} r/sec at 1 watt

THERMAL COLUMN GAMMA EXPOSURE

1. Unit - Los Alamos Homogeneous Reactor; North Thermal Column

2. Power Level - 25 to 30 kw

3. Shielding - 2.45 cm Li

4. Thermal Neutron Flux - Negligible

5. Gamma dose rate - 1.084×10^{-3} r/min at 1 watt

TABLE 2

X-Ray Exposure Data

Dose (r)	W*	Spleen Weight Lost** (Per Cent)	Thymus Weight Lost ** (Per Cent)
50	2	12.6	13.8
75	1	27.0	17.8
100	4	25.2	27.4
125	1	34.9	31.8
175	1	33.0	44.4
200	3	38.6	46.5
275	1	41.5	53.6
300	4	49.3	55.5
400	4	58.6	65.3
500	2	69.0	74.3
600	3	72.7	80.5
700	1	72.8	84.9
800	3	76.4	87.3
900	1	76.6	90.9
1000	3	80.2	90.0

* Weighting factor for number of observations at a particular Dose.

** Mean values of one or more observations at a particular dose.

TABLE 3

Thermal Column Gamma Exposure Data

Dose (r)	Group Spleen Weights	Spleen Weight Lost (Per Cent)	Group Thymus Weights	Thymus Weight Lost (Per Cent)
Control	86.4 ± 4.4*	--	66.4 ± 2.9*	--
100	72.6 ± 2.4	16.2	58.9 ± 2.4	11.3
140	67.2 ± 4.5	22.4	53.5 ± 2.4	19.4
200	55.9 ± 2.1	35.5	48.7 ± 1.5	26.7
280	48.1 ± 2.4	44.5	34.7 ± 2.1	47.7
400	39.6 ± 1.6	54.3	33.5 ± 1.2	49.5
560	30.4 ± 1.5	64.9	27.0 ± 1.5	59.3
790	23.4 ± 1.8	73.0	18.5 ± 1.0	72.1
1000	19.3 ± 0.5	77.7	12.0 ± 0.6	81.9

* Standard error of the mean of each group.

TABLE 4

Mixed Thermal Column Radiation Exposure Data

Exposure Time (Sec)	Group Spleen Weight	Spleen Weight Lost (Per Cent)	Group Thymus Weight	Thymus Weight Lost (Per Cent)
Control	94.1 ± 5.2*	--	87.7 ± 4.4*	--
94	67.1 ± 1.6	24.5	59.0 ± 2.8	31.2
130	60.7 ± 2.5	38.9	52.8 ± 2.1	41.1
165	56.9 ± 1.5	42.7	50.9 ± 1.8	43.3
195	50.7 ± 2.3	48.9	47.4 ± 1.8	47.2
230	47.5 ± 2.2	52.2	36.4 ± 2.0	59.4
282	38.3 ± 3.0	56.9	23.3 ± 1.7	72.8
310	35.3 ± 1.7	64.5	27.2 ± 1.4	69.7
360	32 0 ± 0.9	67.8	19.5 ± 1.2	78.3
420	29.0 ± 0.9	70.8	14.7 ± 0.9	83.6
470	22.3 ± 1.0	74.9	12.9 ± 0.7	84.9
658	16.2 ± 0.7	81.8	9.7 ± 0.4	88.7

* Standard error of the mean of each group.

TABLE 5

Constants and Statistical Parameters Derived from the Data

Radiation Source	Organ	a(1)	V(a)(2)	b(3)	V(b)(4)	S^2(5)
X Ray	Spleen	1.54926	1.17612×10^{-4}	.017828	2.67059×10^{-7}	.00405
	Thymus	1.56716	3.82313×10^{-5}	.015567	6.23434×10^{-8}	.00126
Thermal Column Gamma	Spleen	1.75813	6.57332×10^{-5}	.015668	1.39075×10^{-7}	.00051
	Thymus	1.86987	3.52798×10^{-4}	.014116	6.35039×10^{-7}	.00282
Mixed Thermal Column Radiation	Spleen	1.58559	7.21225×10^{-5}	.014614	1.07184×10^{-6}	.00319
	Thymus	1.63280	2.86030×10^{-4}	.012287	7.81335×10^{-7}	.00317

(1) Intercept constant of the regression line.

(2) Variance (squared standard deviation) of a.

(3) Regression coefficient.

(4) Variance of b.

(5) Squared standard error of estimate of the regression line.

TABLE 6

The Biological Effectiveness of Thermal Column Gamma Compared to 250 KVP X Ray

	Area Method	Point Method	Average
Spleen	0.82	0.80	0.81
Thymus	0.60	0.60	0.60
LD-50	--	--	0.53

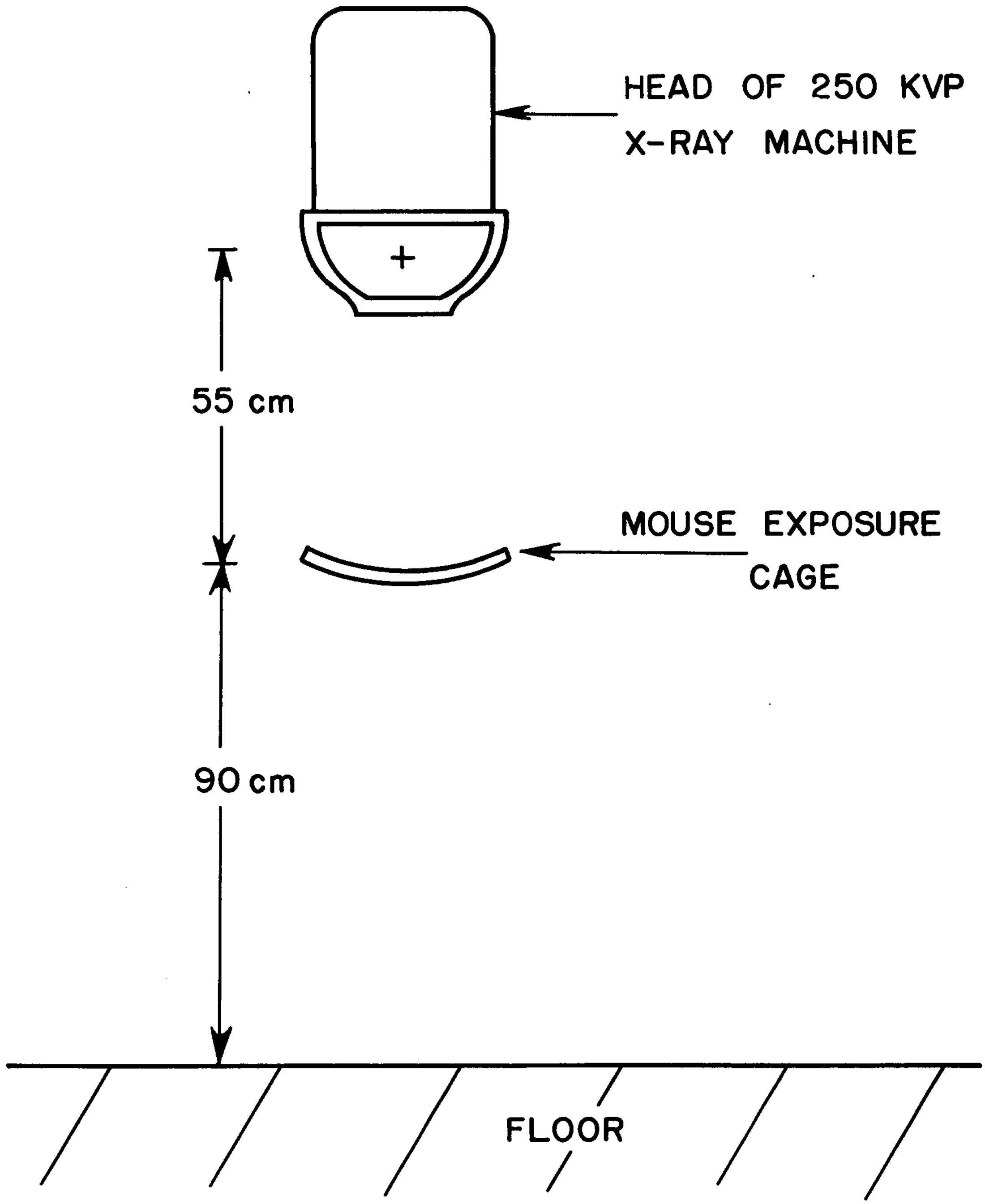

Fig. 1--Characteristics of Apparatus for Exposure of Mice to 250 KVP X Radiation.

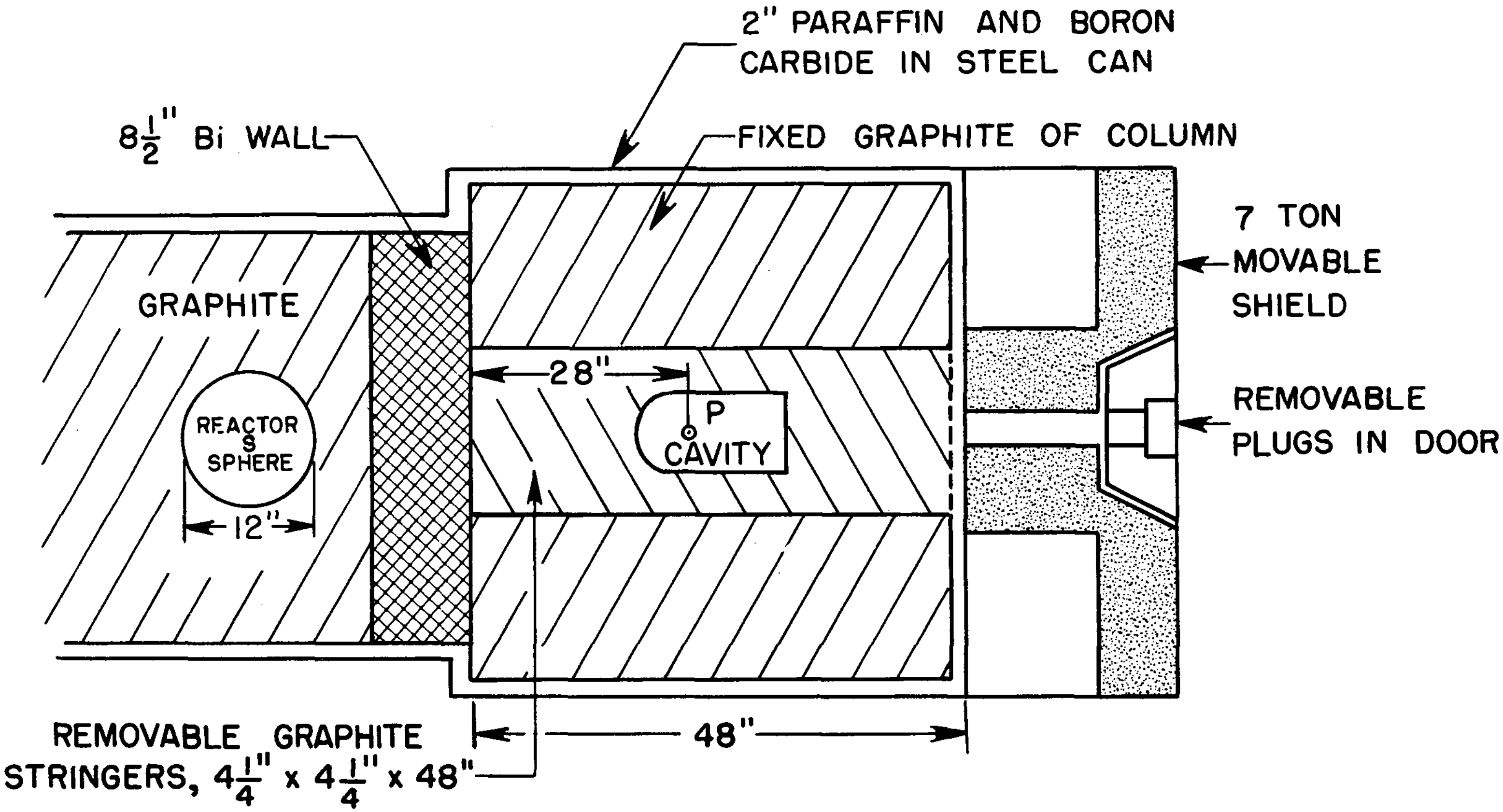

Fig. 2--Essential Characteristics of the Exposure Cavity and the North Thermal Column of the Homogeneous Reactor.

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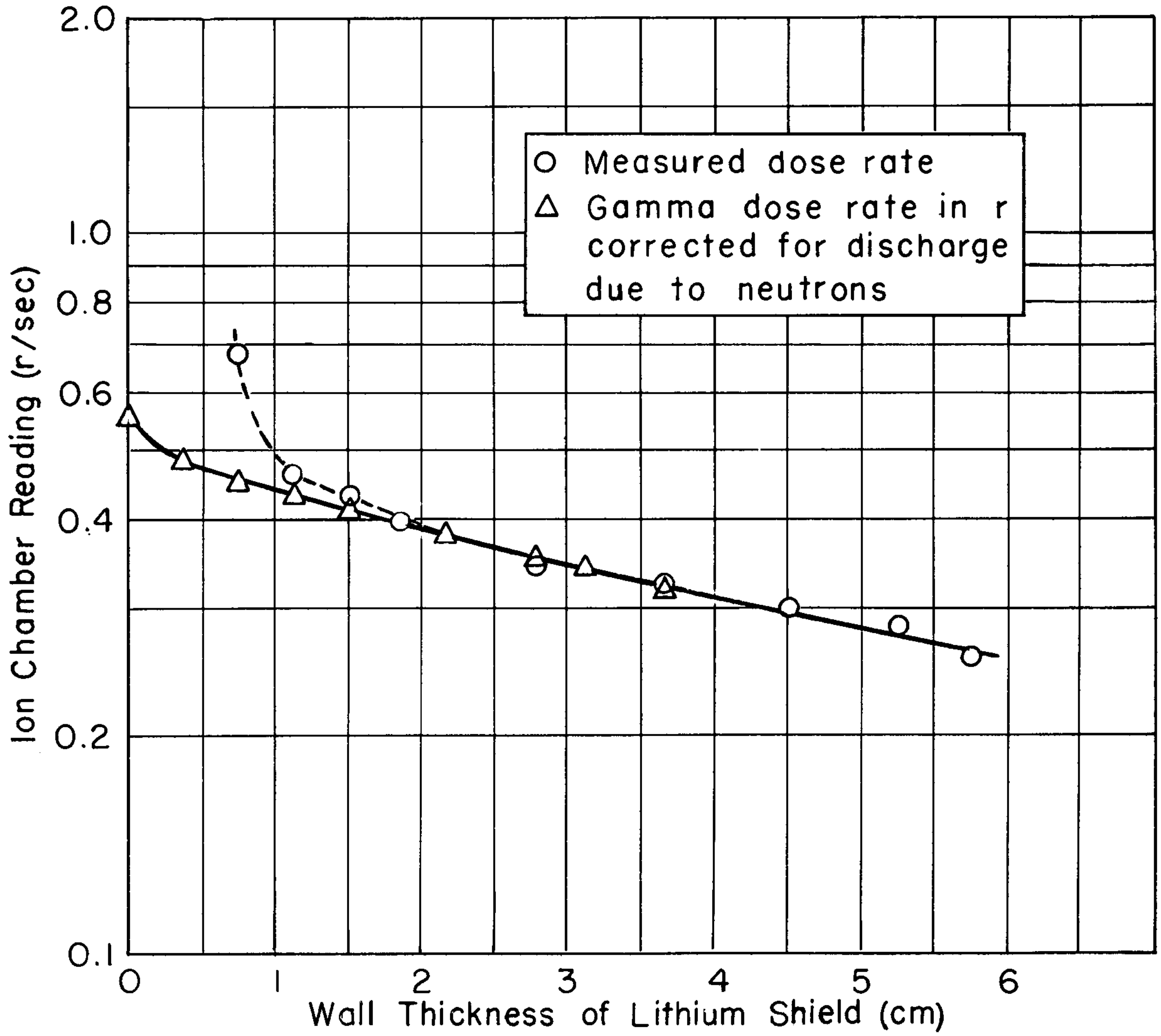

Fig. 3--Absorption of Mixed Radiation of the Thermal Column in Lithium in Poor Geometry.

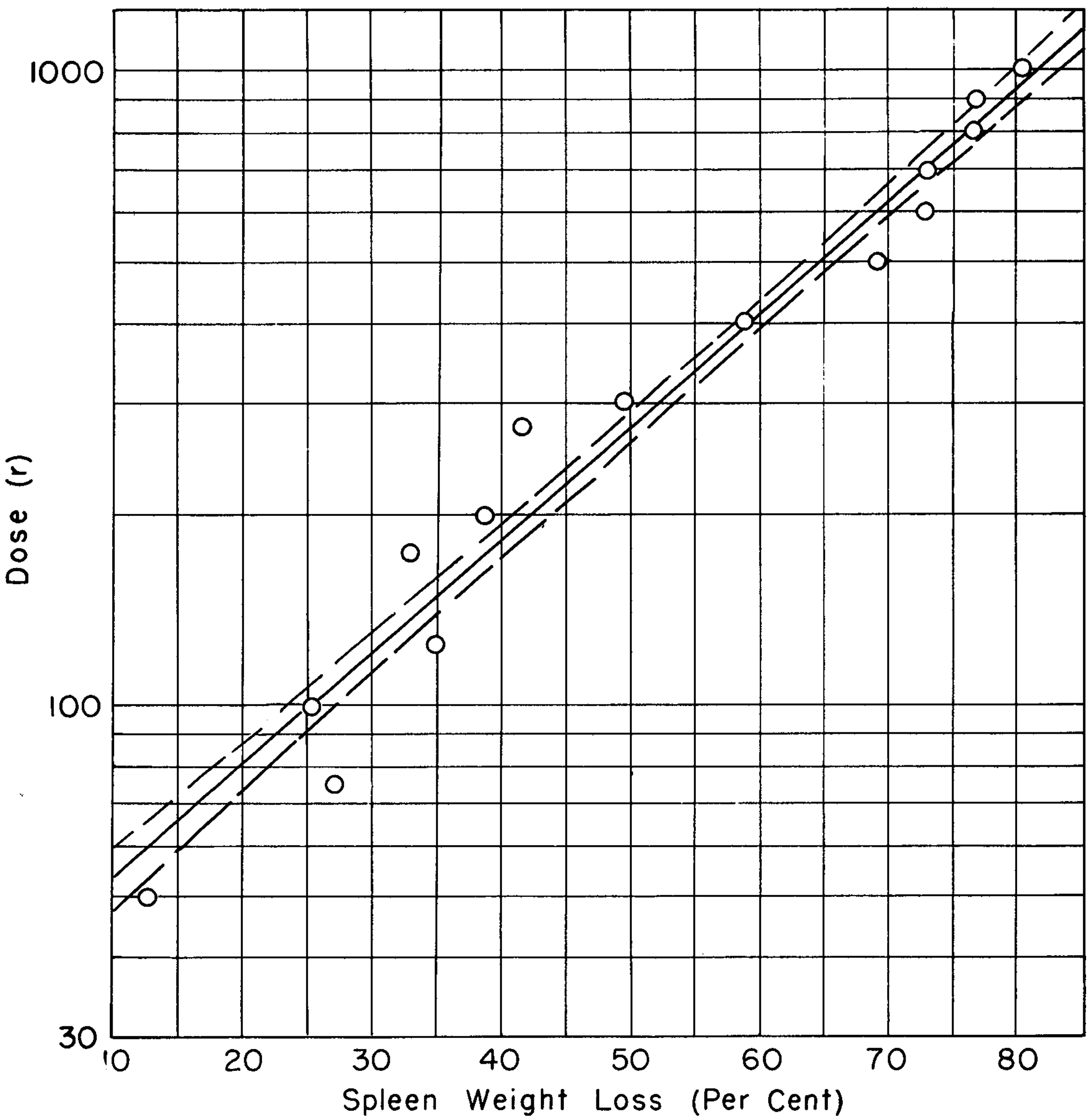

Fig. 4--Splenic Response to 250 KVP X Radiation.

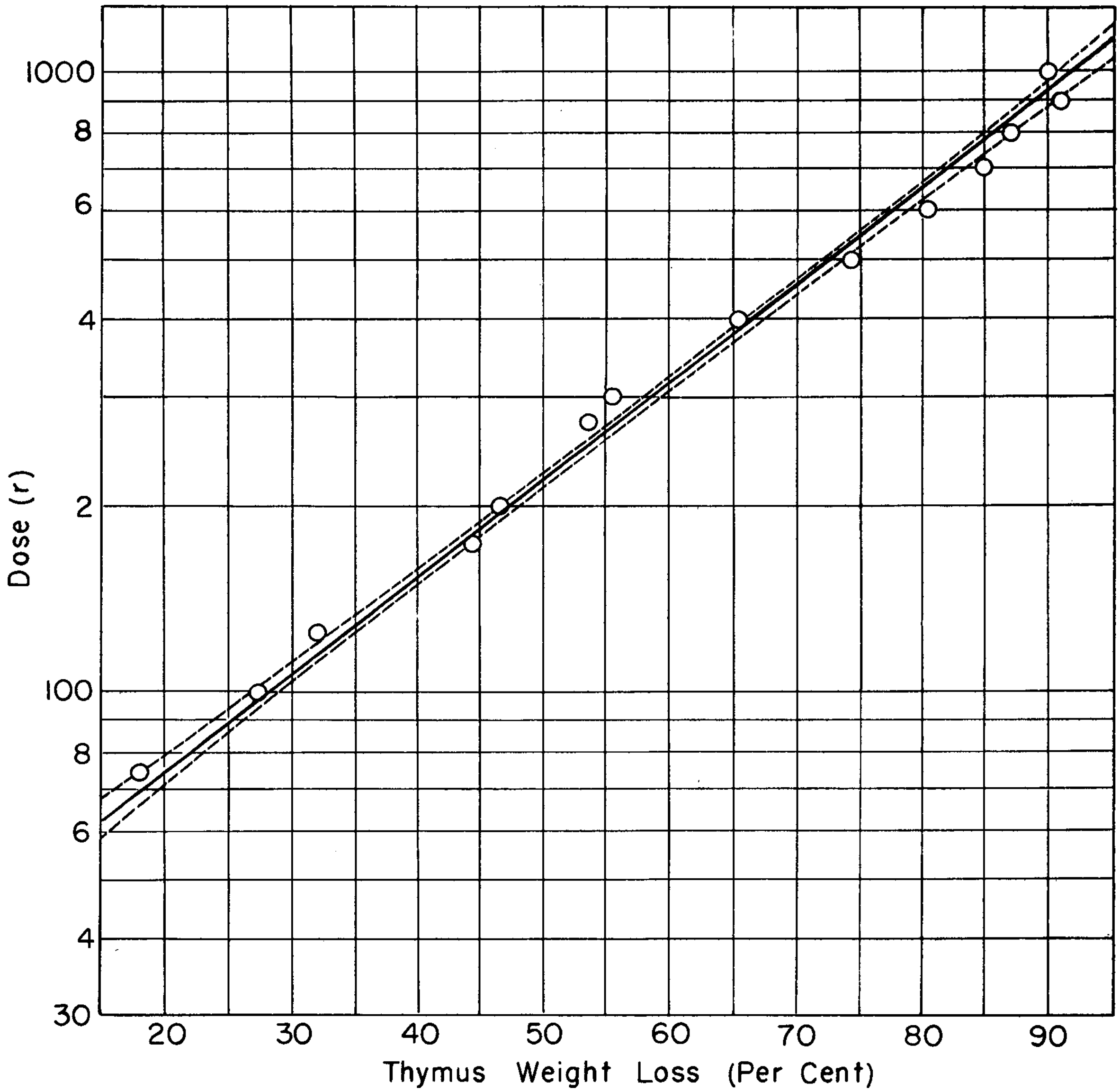

Fig. 5--Thymic Response to 250 KVP X Radiation.

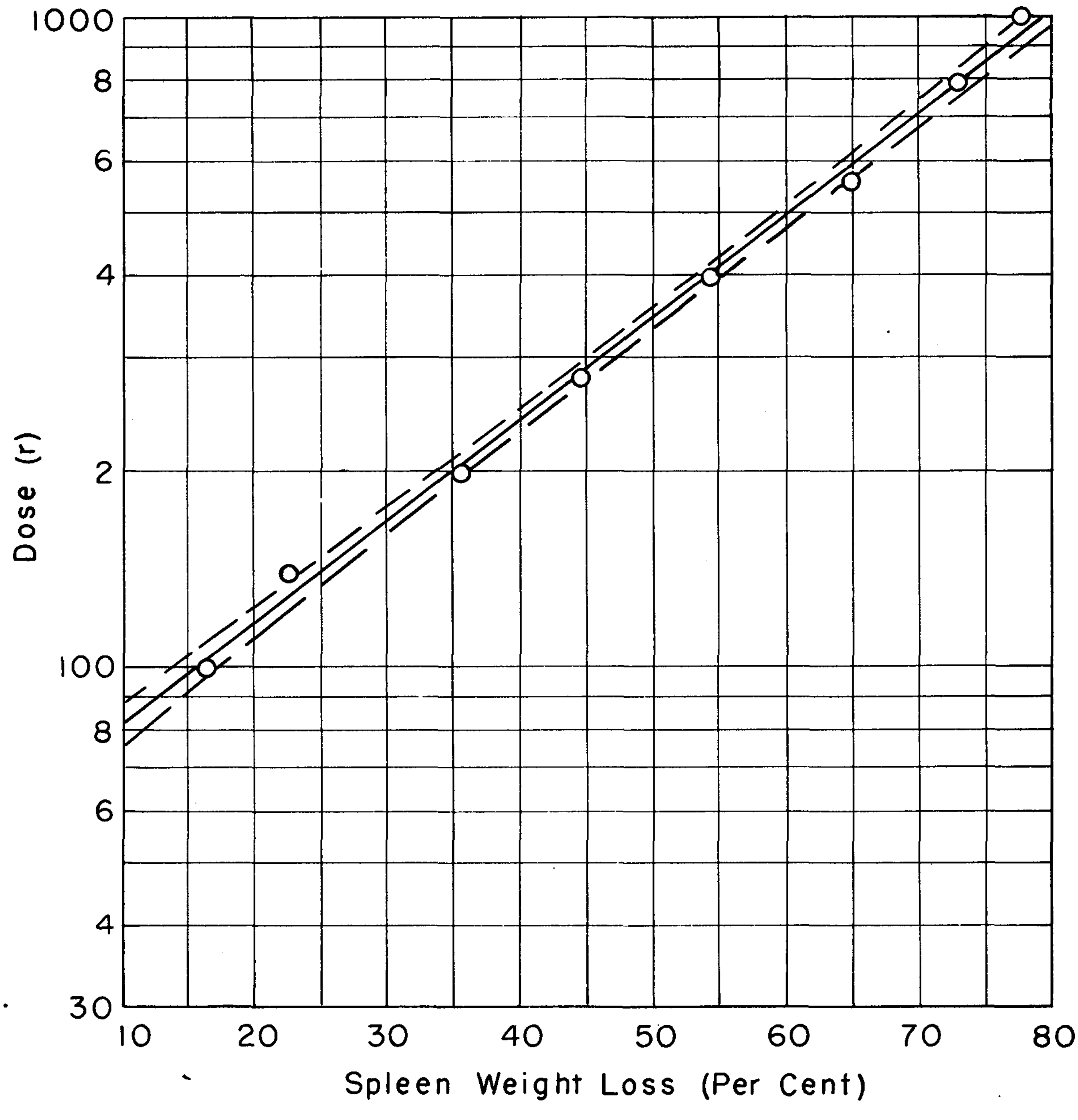

Fig. 6-- Splenic Response to Thermal Column Gamma Radiation.

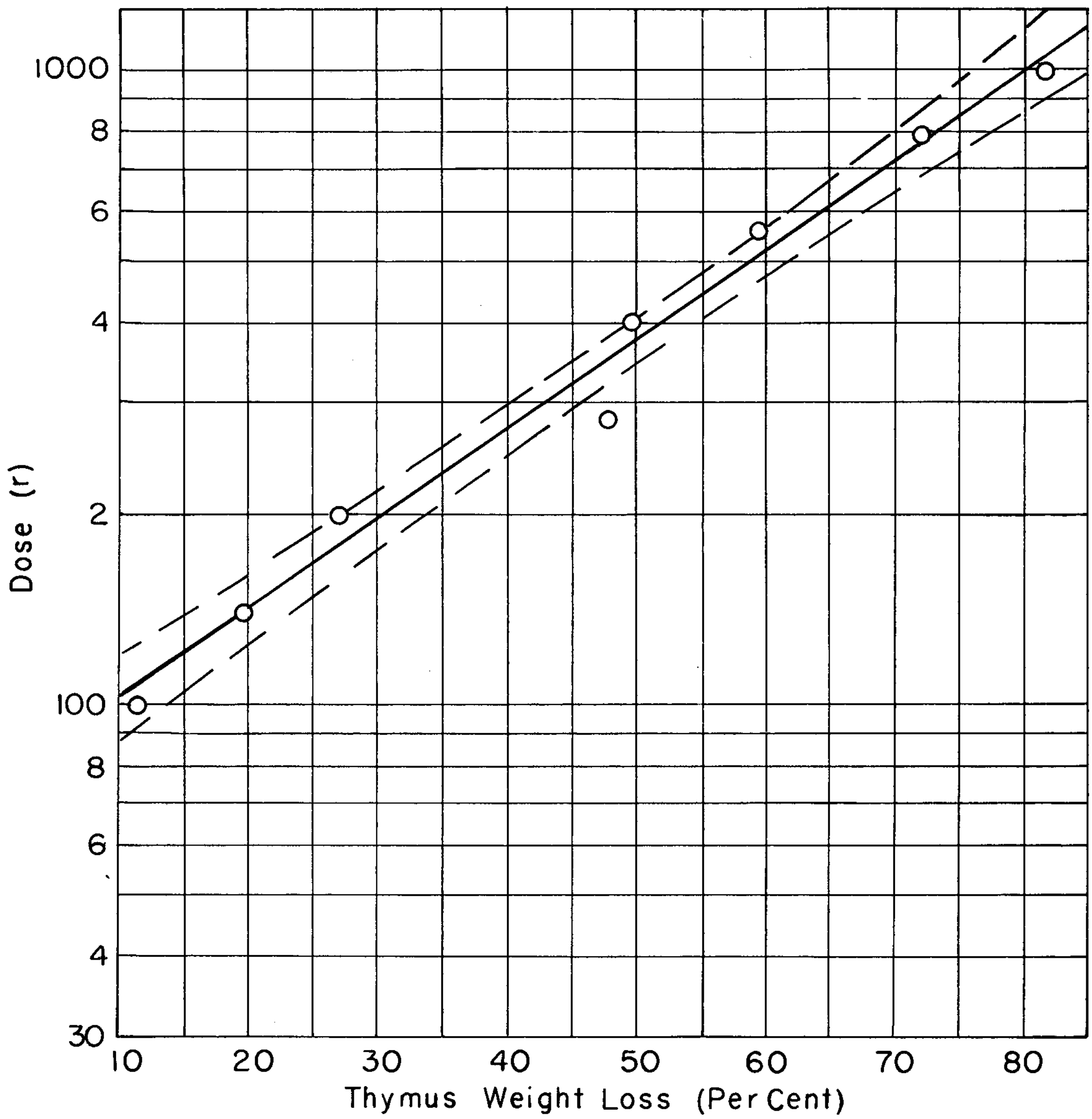

Fig. 7--Thymic Response to Thermal Column Gamma Radiation.

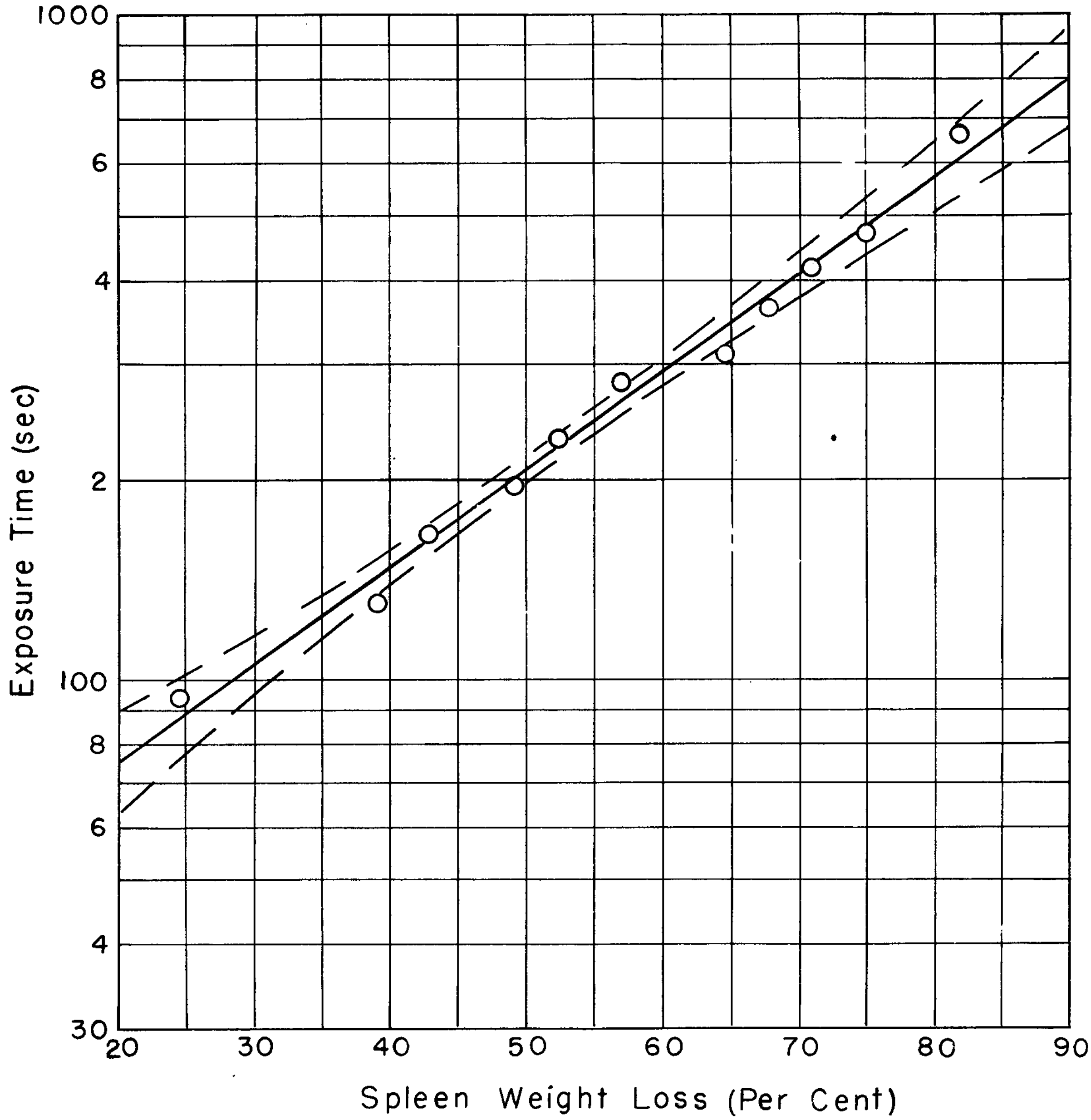

Fig. 8--Splenic Response to the Mixed Radiation of the Thermal Column.

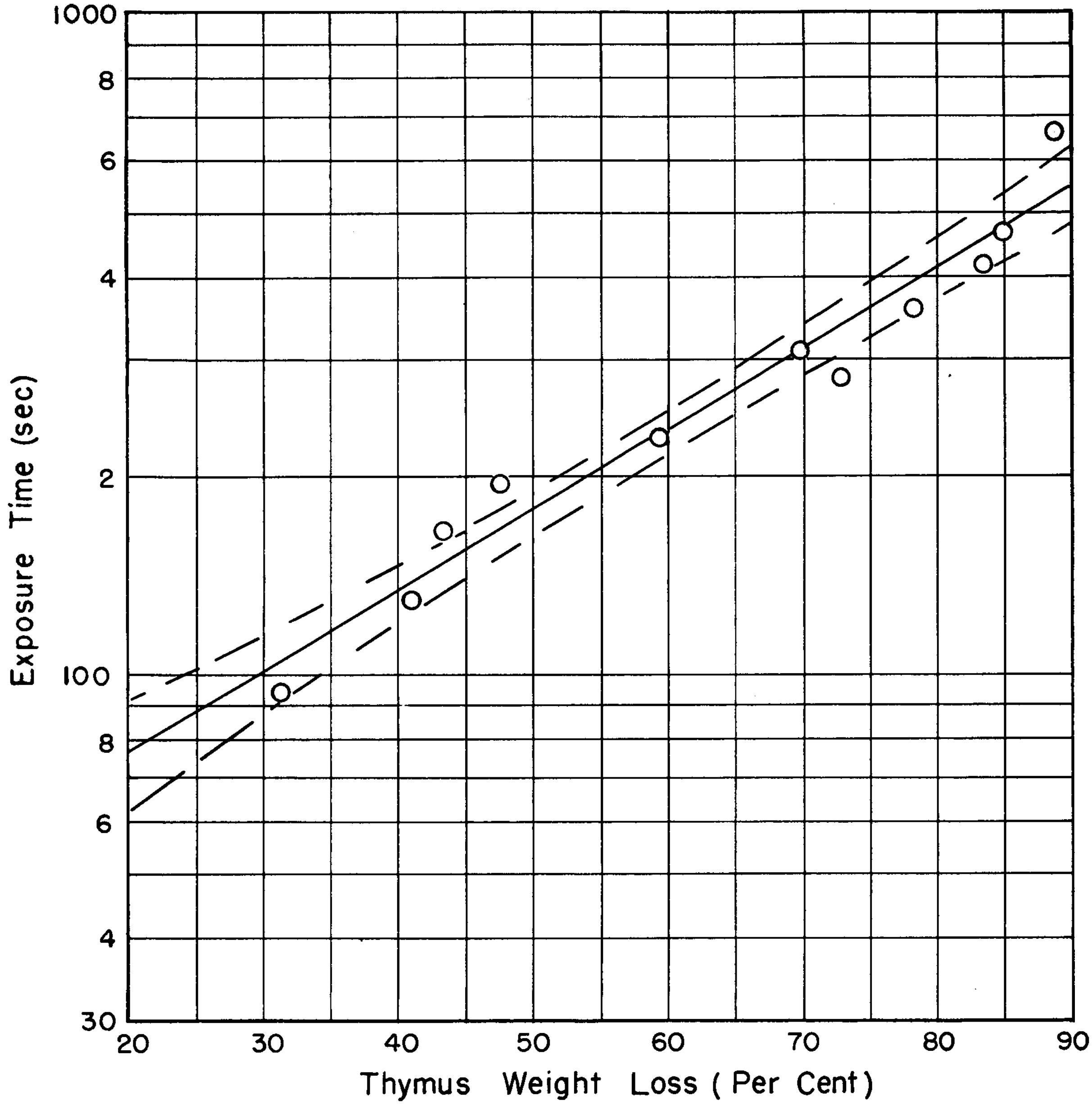

Fig. 9--Thymic Response to the Mixed Radiation of the Thermal Column.