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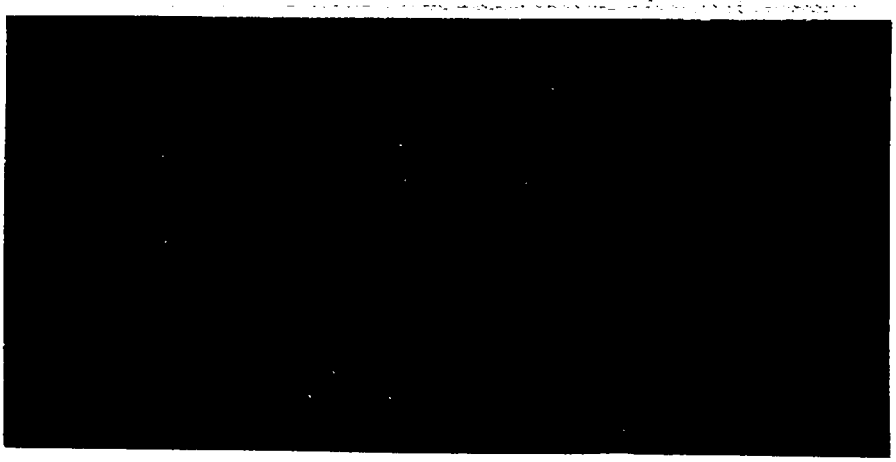

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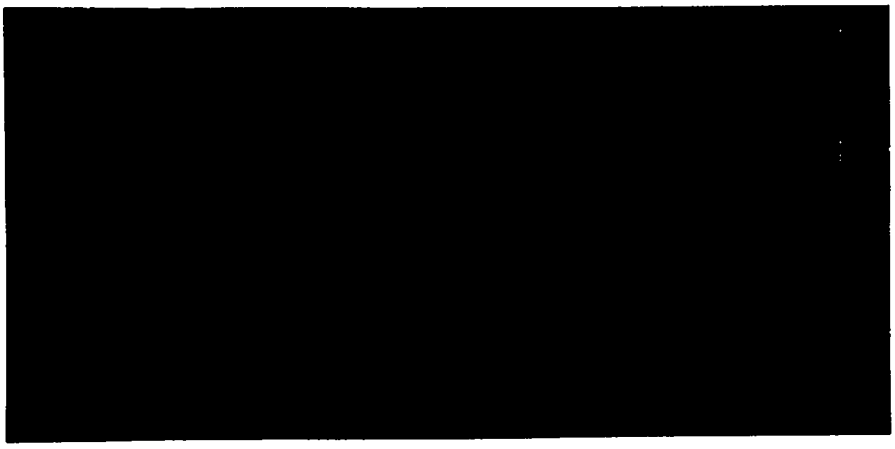

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THE RELATIVE BIOLOGICAL EFFECTIVENESS OF TRITIUM BETA RAYS IN PRODUCING SPLENIC AND THYMIC WEIGHT LOSS IN MICE

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HEALTH AND BIOLOGY

ABSTRACT

One hundred and fifty female CF-1 mice were exposed to total-body radiation by intraperitoneal injection of varying doses of tritium oxide followed by addition of tritium oxide to the drinking water to maintain approximately the desired level in the body water over an exposure period of 120 hours.

One hundred and fifty mice were exposed over the same period of time to varying doses of radium gamma radiation. At the conclusion of the exposures the percentages of splenic and thymic weight loss (based on control weights) were calculated and compared as a function of dose. The beta radiation from tritium was found to be approximately 1.3 times as effective as the gamma radiation in producing splenic atrophy and approximately 1.5 times as effective in producing thymic atrophy. Assuming equivalence of r, rem, and rep of radium gamma rays the average RBE of tritium beta radiation was 1.4 for the biological test systems used.

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1. Introduction

The increasing use of tritium (H^3) in investigations in the fields of biology, medicine, and atomic energy has brought about an increasing interest in expanding present knowledge of the potential radiation hazard of this isotope.

In the evaluation of the hazard from ionizing radiations it is necessary to take into account whether or not the particular type of radiation in question is more or less effective than gamma or X rays (for which tolerance values are well established) in producing biological effects. The ratio of biological effect (expressed in rem) to the energy imparted to the test system (in rep) is known as the relative biological effectiveness, or RBE.

Tritium decays with a half life of 12.5 years by the emission of beta particles having an average energy of ~6 kev.[1] Although there are considerable differences of opinion as to the exact extent of the relationship it is generally agreed that radiations which produce high linear ion densities in tissues are somewhat more effective in producing biological effects than radiations which produce relatively low ion densities. It might be expected, then, that the tritium beta particle would be more effective than gamma rays in producing biological effects. Jennings and Brues[2] in a study of toxicity of tritium oxide (HTO) showed that the RBE of tritium beta radiation was slightly greater than 1. Furchner and Storer[3] studied the effect of tritium beta radiation on the uptake of Fe^{59} by red cells in rats and found an RBE of 1.6.

Any value for RBE is strictly valid only for the particular biological test system by which it was determined. Since various test systems may give different values for the RBE it is desirable to determine values for relative effectiveness by measuring a variety of biological effects. On the basis of values so obtained it is then possible to establish a rough overall value for the RBE with considerably more confidence.

The present study was undertaken to determine the RBE of the beta particles from tritium in producing atrophy of the spleen and thymus of mice.

2. Spleen-Thymus Weight Loss as a Biological Indicator, and Statistical Methods

The details of this method and its validity as a biological indicator have been established in a number of reports from this laboratory.[4] Briefly, the details of the method are as follows. Mice are exposed to various doses of radiation (of any type) in the range of 50 to 1,000 rep. Five days from the beginning of the radiation exposure the mice are killed and the wet weight of the spleen and thymus determined. When the change in weight of these organs is expressed as percentage of weight loss as compared to the organ weights of unirradiated control animals the relationship between weight loss and radiation dose can be expressed mathematically by the equation:

$$y = a + b \log x$$

where y = percentage of weight loss of the spleen or thymus, x = dose in r or rep, $a = \bar{y} - b\bar{x}$ (where $\bar{y}$ and $\bar{x}$ are mean values of x and y), and b = slope of the regression line.

In comparing the effectiveness of dissimilar radiations it is possible to determine the relative effectiveness over the entire range of dosages employed by substituting in the formula:

$$\log E = \frac{a_x - a_y}{b}$$

where E = relative effectiveness, a_x = intercept constant for baseline radiation, a_y = intercept constant for radiation being investigated, and b = common slope of the regression lines.

It is obvious that in order to solve the above equation it must be possible to obtain a value for b which is common to the equations derived for both types of radiation. When a homogeneous population of mice is used and the radiations exert their effects by similar mechanisms, the slopes (b_x and b_y) are rarely signficantly different as determined by the "t" test. A common value b can therefore be obtained by weighting the values b_x and b_y by the inverse of the variances. A more complete discussion of these statistical concepts is given in a report by Harris and Brennan.[5]

3. Experimental Methods

3.1 Care and Handling of Animals

A total of 330 female CF-1 mice 5 to 7 weeks of age were used in the present study. Prior to radiation exposure the animals were randomly distributed into 11 groups of 30 mice each. During the randomization procedure any mice which deviated in weight by more than 2 standard deviations from the previously established average weight were discarded. The mice were maintained on Purina laboratory chow and water ad libitum. In the case of the mice exposed to tritium radiation, tritium oxide (HTO) in varying amounts was added to the drinking water.

3.2 Exposure to Radium Gamma Radiation

Five group of 30 mice each were exposed to gamma radiation continuously for 5 days. The source used contained 493.5 mc of radium in a Monel metal container. The source was calibrated by the National Research Council of Canada.

The mice were exposed in cages curved as arcs of circles 28, 36, 41, 51, and 69 cm in radius. The cages themselves were 36 in. long, 3 in. high, and 1 in. wide. The cages were placed in different planes with respect to one another so that no shielding of one group of mice by another could occur. The narrow width of the cages allowed freedom of movement by the mice but prevented shielding of one mouse by another.

Dose rate determinations at the locations of the various cages were made repeatedly during the radiation exposure with Victoreen thimble chambers.

A group of 30 mice were similarly caged for 5 days but received no radiation. These mice served as controls.

The radiation doses and numbers of mice used are summarized in Table 1.

3.3 Exposure to Tritium Beta Radiation

Tritium oxide distributes itself homogeneously throughout body water.[6] This property makes it possible to deliver total body radiation by using the isotope as an internal emitter. Since the tritium oxide is distributed in the body water it is obvious that the concentration in an animal will come to equilibrium with the concentration in the ingested water. The desired initial concentrations were obtained by intraperitoneal injection of HTO into the mice. The level was then maintained approximately constant by adding tritium oxide to the drinking water. To compensate for the water formed by metabolism it was necessary to supply drinking water having 4/3 the desired tritium concentration in the body fluids of the animals.[6]

Four groups of 30 mice each were maintained at body water concentrations of tritium of 108, 136, 209, and 348 μc/ml, respectively, for a period of 5 days. Ten mice from each group were housed together in standard metabolism cages and the pooled urine samples were collected daily. The urine samples were assayed for tritium concentration by the method previously described by Pinson.[6] The radiation doses delivered to the various groups of mice were calculated from the values for average tritium concentration in the body water using the following formula:

$$\text{rep} = \frac{(A)\,(D)\,(E)\,(T)\,(G)}{R}$$

where A = activity in μc/ml in body fluids, D = disintegrations/sec/μc (3.7×10^4), E = average energy of tritium beta particles in ev (6×10^3), T = exposure time in sec (5 days = 4.32×10^5 sec), G = gm of water/gm of tissue (0.75), and R = ev/rep/gm of tissue = 5.8125×10^{13}. The calculated doses were 134, 158, 265, and 376 rep, respectively, for each of the exposure groups. Data on concentrations in body water, urine, etc., and the dosages delivered are summarized in Table 2.

3.4 Measurement of Splenic and Thymic Weight Loss

Splenic and thymic weight loss as an index of radiation effect is least variant at 5 days from the beginning of radiation exposure.[4] Five days from the start of the exposure the mice were killed with ether and the wet weights of the spleen and thymus determined. Weights which deviated from the mean for each group by amounts determined by the Chauvenet Criterion

were excluded in the final analysis of the data. The data on weight loss for each group are summarized in Table 3.

4. Results

Increasing doses of total-body gamma radiation from radium or total-body beta radiation from tritium produced increasingly greater decreases in splenic and thymic weights (Table 3).

If the data are plotted on semilog paper as percentage of weight loss versus the logarithm of dose, a straight line of the type $y = a + b \log x$ gives the best fit for the experimentally determined points. The data for splenic weight loss are plotted in Fig. 1 and the data for thymic weight loss are plotted in Fig. 2. To facilitate analysis of the data the spleens and thymuses are considered separately.

For the spleen the two regression lines (calculated by the least squares method) may be expressed as follows:

Ra γ-radiation, $y = -93.98 - 57.1 \log x$

H^3 β-radiation, $y = -139.86 - 79.2 \log x$

and for the thymus:

Ra γ-radiation, $y = -97.37 - 63.30 \log x$

H^3 β-radiation, $y = -118.57 - 76.96 \log x$

where y = percentage of weight loss and log x = log dose in r or rep.

Determination of the significance of the difference in the slopes of the two regression lines indicated that there was an 80% probability that the difference was due to chance and it was concluded that the slopes were not significantly different. A common slope was obtained by weighting each slope by the inverse of its variance and taking the average. New values for the intercept constants a were then calculated using the common value for b (the slope). The recalculated regression lines were as follows:

For the thymus:

Ra γ-radiation, $y = -104.83 - 66.28 \log x$

H^3 β-radiation, $y = -93.67 - 66.28 \log x$

For the spleen:

Ra γ-radiation, $y = -96.29 - 58.04 \log x$

H^3 β-radiation, $y = -90.54 - 58.04 \log x$

The relative effectiveness or relative potency of tritium beta radiation in terms of radium gamma rays was calculated by using the general formula for comparing the effectiveness of dissimilar radiations given on page 5. Substitution of the constants for the adjusted slopes given above in the equation gave a value of 1.26 ± 0.08 for the RBE of H^3 beta rays when splenic weight loss was used as the end point, and a value of 1.47 ± 0.12 for thymus, assuming 1 r of radium gamma radiation = 1 rem = 1 rep.

5. Discussion

On an energy (rep) basis, the beta radiation from tritium was found to be 1.3 (spleen) to 1.5 (thymus) times as effective as radium gamma radiation in producing splenic and thymic atrophy in mice. These results are in fair agreement with the value of slightly greater than 1[2] and 1.6[3] reported by other investigators using different biological test systems.

The linear ion density in tissue from the gamma radiation is roughly 15 ionizations/μ. For the 6 kev tritium beta particles the ion density is approximately 150 ionizations/μ.[7]

The present study shows that in this range of ion densities an increase in ion density of a factor of 10 increases the RBE by less than a factor of 2. This result suggests that predictions of RBE from strictly physical considerations of the ion density is a procedure subject to considerable error. This conclusion becomes particularly apparent in view of other work from this laboratory which indicates that the RBE of 0.6 Mev protons is about 1.3 - 2.2 times that of gamma rays even though there is about a factor of 100 difference in linear ion density.[8,9]

The present maximum permissible body burden of tritium for man is 10 mc.[10] This value is based on the assumption of an RBE of 1. On the basis of the present study it is concluded that the RBE for mammalian tissue is not signficantly different from unity to necessitate a change in the tolerance value. If, however, the observed RBE is taken into consideration the maximum permissible body burden for tritium is $\sim$7 mc.

6. Conclusions and Summary

When compared with radium gamma radiation the RBE of tritium beta rays was 1.3 and 1.5 for production of atrophy of the spleen and thymus, respectively. The percentage of weight loss of both organs varied directly with the logarithm of the dose. Since the two RBE results vary less than 2 standard deviations from the mean, the differences may be considered as not significant and an average of 1.4 may be accepted as the relative biological effectiveness of tritium beta rays when compared to radium gamma radiations using splenic and thymic weight loss in CF-1 mice as the biological index of radiation effect.

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TABLE 1

CONDITIONS FOR EXPOSURE OF CF-1 FEMALE MICE TO RADIUM GAMMA RADIATION

Group	Source-Cage Distance (cm)	Dose Rate (r/hr)	Corrected Dose Rate (r/hr)	Total Dose 120 hr (r)
1	28	3.620	4.706	564.7
2	36	2.562	3.331	399.7
3	41	2.177	2.830	339.6
4	51	1.539	2.001	240.1
5	69	1.122	1.459	175.1

TABLE 2

CONDITIONS FOR EXPOSURE OF CF-1 FEMALE MICE TO TRITIUM BETA RADIATION

Group	HTO Injected per 22 gm (μc)	Expected Conc. in Body Water (μc/ml)	Conc. in Drinking Water (μc/ml)	Conc. in Urine (μc/ml)	Dose (rep)
1	5742.0	348.0	464.0	304.0	376.2
2	3448.5	209.0	278.8	214.4	265.3
3	2376.0	136.0	181.2	127.2	157.9
4	1782.0	108.0	144.0	108.4	134.1

TABLE 3

EFFECTS OF RADIUM GAMMA AND TRITIUM BETA RADIATIONS ON PERCENTAGE OF WEIGHT LOSS OF SPLEENS AND THYMUSES OF CF-1 FEMALE MICE

Spleen

Group	Dose Ra γ-rays (r)	Weight Loss (%)*	Group	Dose H^3 β-rays (rep)	Weight Loss (%)*
1	564.7	62.5	1	376.2	62.0
2	399.7	55.5	2	265.3	54.3
3	339.6	51.5	3	157.9	38.5
4	240.1	40.5	4	134.1	24.3
5	175.1	34.6			

Thymus

Group	Dose Ra γ-rays (r)	Weight Loss (%)*	Group	Dose H^3 β-rays (rep)	Weight Loss (%)*
1	564.7	78.4	1	376.2	77.6
2	399.7	65.5	2	265.3	70.2
3	339.6	63.6	3	157.9	54.2
4	240.1	50.6	4	134.1	41.4
5	175.1	46.8			

* Based on wet organ weights of control animals.

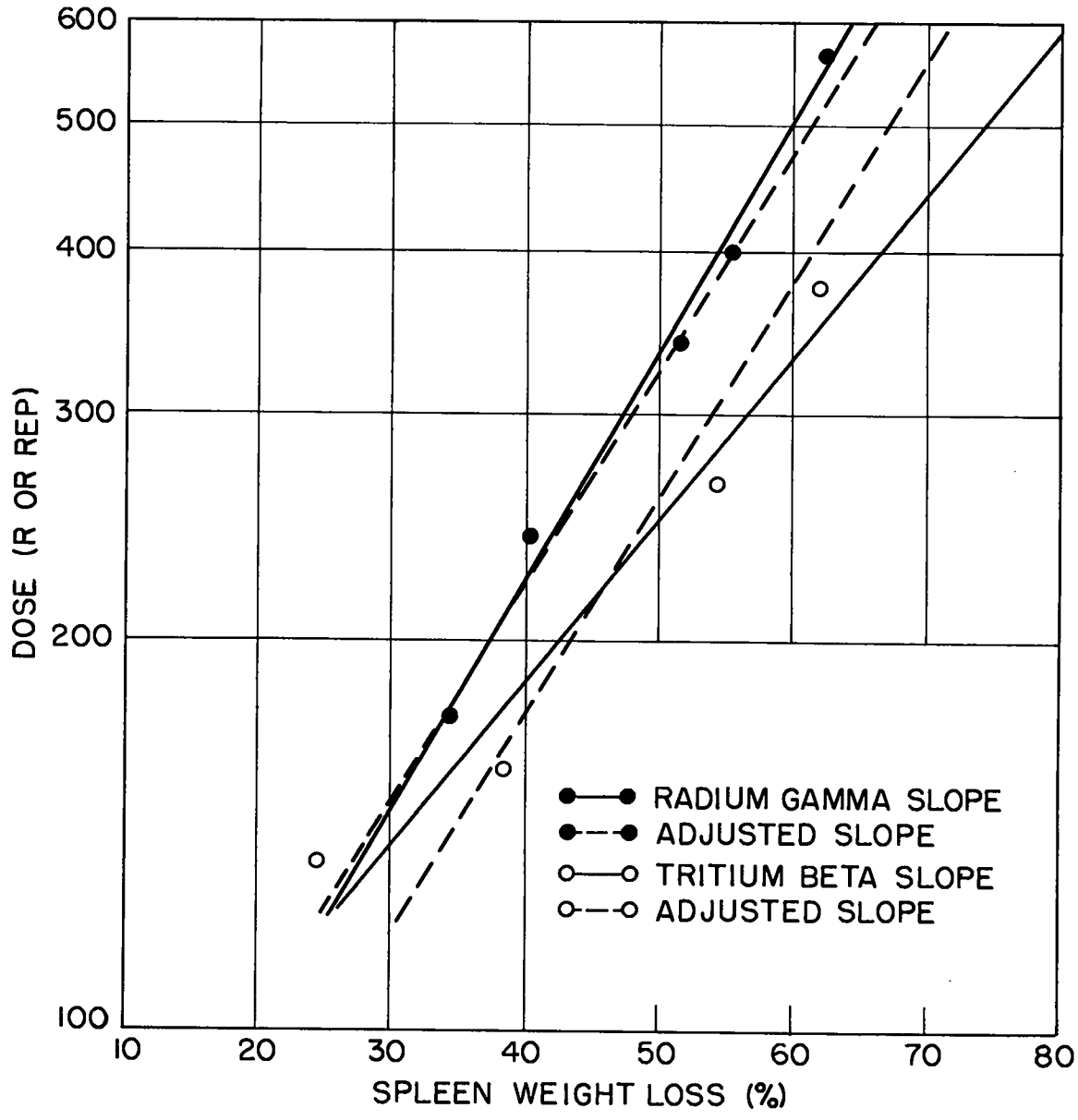

Fig. 1 Percentage of spleen weight loss as a function of dose of radium gamma and tritium beta radiations.

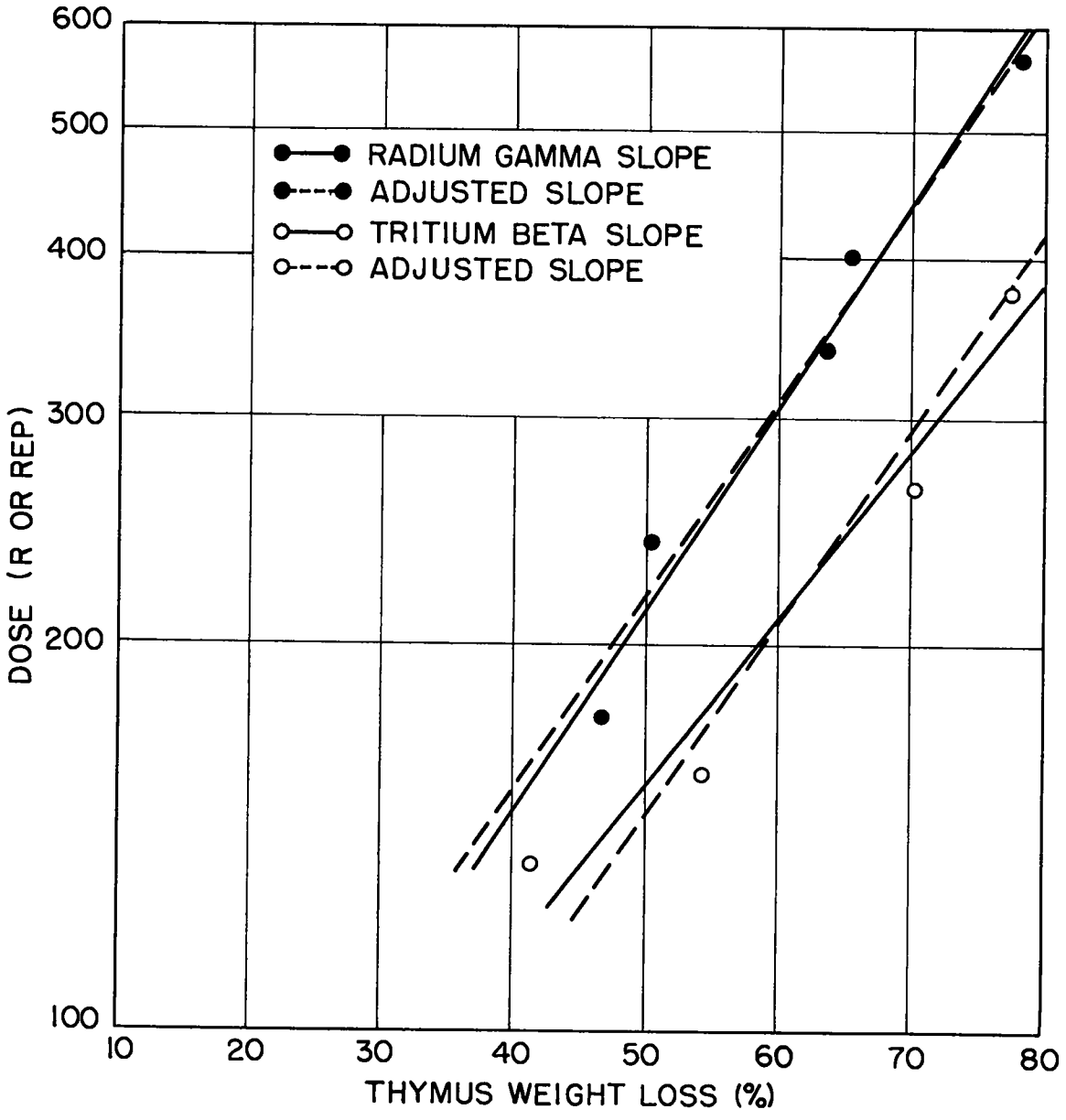

Fig. 2 Percentage of thymus weight loss as a function of dose of radium gamma and tritium beta radiations.