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THE RELATIVE BIOLOGICAL EFFECTIVENESS OF TRITIUM IN DEPRESSING IRON UPTAKE IN RATS

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ABSTRACT

Rats were exposed to total-body beta radiation by intravenous injections of tritium oxide. Concentrations of tritium oxide in the body water were maintained by appropriate concentrations of tritium in drinking water. On the second day of exposure $Fe^{59}Cl_3$ was injected intravenously. Bone marrow damage from tritium beta radiation was measured by comparing the radio-iron uptake of the red blood cells of the exposed animals with that of unirradiated controls. A parallel experiment was performed in which rats were exposed to the gamma radiation of Co^{60} over the same time period and at essentially the same dose rates delivered by the tritium beta radiation. The percentage of normal iron uptake varied inversely with the log of the dose in both experiments. Comparison of the effects of the two radiations gave an RBE of 1.59 for the beta radiation of tritium compared with the gamma radiation of Co^{60}.

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1. Introduction

The increased use of tritium in biological and medical investigations has made consideration of its radiation tolerance value of considerable practical importance. Tolerance values for various types of radiation are usually based on the well established value of 0.3 r per week of gamma or X radiation, with a safety factor added to allow for any difference between the biological effectiveness of the radiation in question and X or gamma rays. This difference in effectiveness is known as the relative biological effectiveness or RBE. Taking 1 rep = 93 ergs/gm of tissue and assuming an RBE of 1 for the tritium beta particle compared to X or gamma rays, a 70 kg man is receiving the permissible dose of 0.3 rep per week when the body contains 10 mc of tritium.

At the first tripartite tolerance conference[1] held at Chalk River in 1949, a safety factor of 10 was introduced and the best estimate of a safe dose of tritium was given as 1 mc. In 1953, however, the National Bureau of Standards Handbook 52[2] gave 10 mc as the maximum amount of tritium permissible in the body. As more information is obtained on the RBE of tritium beta rays it may be possible to establish the relative hazard from exposure to this material with more confidence.

In a study of the toxicity of tritium oxide, Jennings and Brues[3] indicated that the RBE of the beta radiation was slightly greater than 1, although no exact figure was given. Snyder and Kisieleski[4] determined the RBE of the beta particle of Na^{24} to be 1.4. It has been suggested that the RBE of any ionizing radiation is dependent on the specific ionization of the radiation. If this is true then a considerable difference in the RBE of tritium and Na^{24} would be anticipated since the average energy of the Na^{24} beta particle is approximately 90 times that of the tritium beta particle and specific ionization is inversely related to energy.

The present study has determined the relative effectiveness of tritium beta particles as compared to Co^{60} gamma radiation in inhibiting bone marrow function in rats.

2. Radio-Iron Uptake as a Biological Indicator

It has been shown previously that the degree of depression of radio-iron (Fe^{59}) uptake by red blood cells bears a quantitative relationship to radiation dosage.[5,6] This relationship may be indicated by the linear equation:

$$Y = a + b \log X,$$

where Y = radiation effect (in this case percentage of normal Fe^{59} uptake),

a = the intercept constant,

b = the slope constant, and

X = radiation dose.

Depression of Fe^{59} uptake is generally believed to measure the degree of damage to erythropoietic tissue by ionizing radiation and has been used by other investigators.[5]

3. Experimental Methods

3.1 Care and Handling of Animals

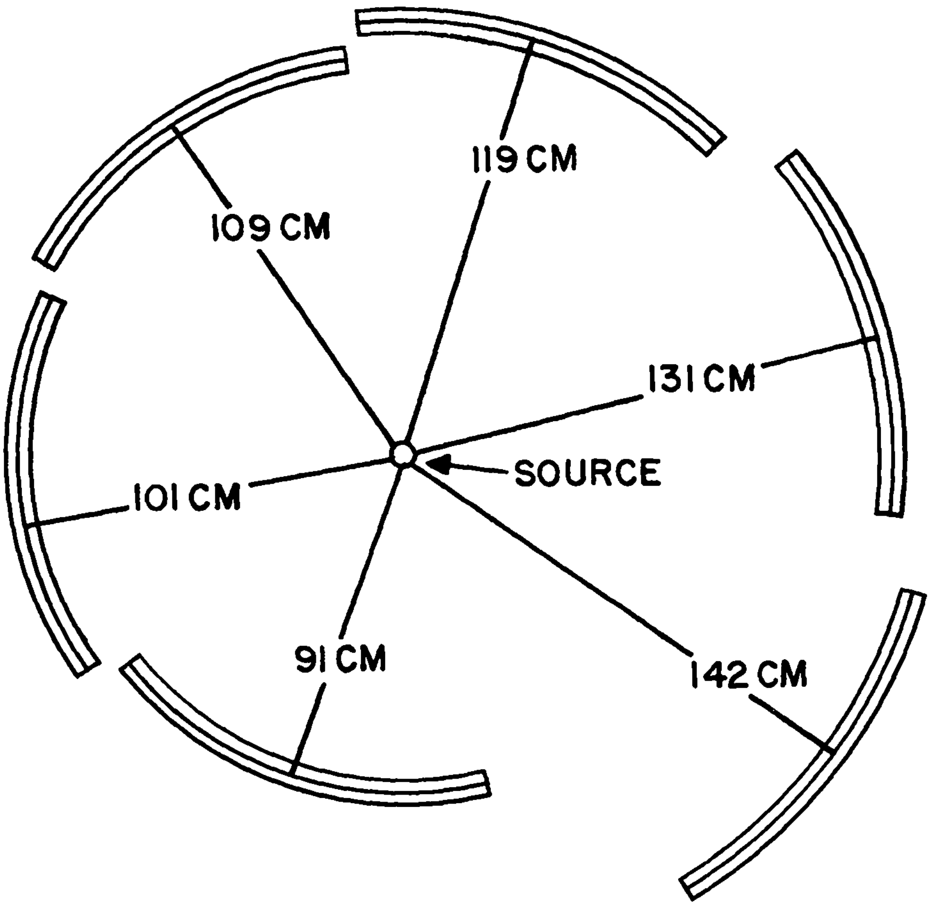
One hundred and fifty-four male Sprague-Dawley rats, 3 to 4 months of age and weighing 190 to 220 gm, were used in the present study. Before radiation exposure the animals were maintained 3 or 5 to a cage and given water and Purina laboratory chow ad libitum.

Seven groups of 10 animals each were exposed to Co^{60} radiation and seven groups of 12 animals each were exposed to tritium beta rays (the tritium being used as an internal emitter). The rats were divided into the various groups by the use of random number tables.

3.2 Exposure to Co^{60} Gamma Radiation

The source used for gamma ray exposures consisted of 5 curies of Co^{60} contained in a Lucite cylinder 3 cm long and 1 cm in diameter with a wall thickness of 0.75 mm. Exposure cages, shaped to fit arcs of circles of appropriate radii, were fixed at 91, 101, 109, 119, 131, and 142 cm from the source. The cages were so constructed that the rats necessarily remained tangent to the radiation source, and shielding of one rat by another was negligible. The cage dimensions and animal-source relationships are shown in Fig. 1.

Dosage determinations were made with Victoreen 100 r thimble chambers exposed to the source before, concomitant with, and following, exposure of the animals. The average values found from four 16 hr exposures of the thimble chambers were used as the basis for dosage calculations.



CAGE DIMENSIONS:
3 FT ARC
5 CM WIDE
8 CM HIGH

Fig. 1. Cage-source relations for Co^{60} gamma radiation exposure.

The total doses received by the various groups of rats were calculated to be 302, 354, 404, 468, 579, and 632 r (Table 1). The total doses of gamma radiation were delivered over a 5-day time interval. Throughout the exposure period all the rats had free access to Purina laboratory chow and water.

TABLE 1

EXPOSURE CONDITIONS AND EFFECT OF Co^{60} GAMMA RADIATION ON PERCENTAGE OF NORMAL IRON UPTAKE BY THE RED BLOOD CELLS OF RATS

Group	Source-cage distance, cm	Dose, r	Normal Fe^{59} uptake, percent(*)	Standard error of mean
1	91	632	25.7	7.8
2	101	579	29.4	14.5
3	109	468	43.7	21.4
4	119	404	50.8	20.7
5	131	354	54.0	22.4
6	142	302	59.7	14.3

(*) Iron-uptake, control = 59.5 ± 9.06 percent.

3.3 Exposure to Tritium Beta Radiation

Since tritium oxide (HTO) distributes itself homogeneously throughout body water, it was possible to deliver what amounted to total-body beta radiation by using the oxide of the isotope as an internal emitter. Initial concentrations of HTO in the body water, obtained by intraperitoneal injection, were maintained at fairly constant levels by giving additional amounts of HTO in the drinking water. In this way it was possible to balance the intake and excretion of the isotope. About 30 percent of the water intake in mice has been shown to come from food and absorption from the air,[7] and presumably, approximately the same value applies to rats. By giving drinking water containing four-thirds the concentration of tritium desired in the body water it was possible to maintain the body water concentration of tritium at approximately the desired level.

Six groups of 12 rats each were injected intraperitoneally with sufficient HTO to obtain tritium concentrations of 254, 293, 342, 368, 413, and 514 μc/ml of body water. After injection the animals were placed in individual metabolism cages and given Purina laboratory chow, and water containing appropriate concentrations of HTO, _ad libitum_ for 5 days. Simultaneously, 12 uninjected animals were kept as controls. Urine samples were collected daily and assayed for tritium by the method previously described by Pinson.[7] Since the tritium in the urine is in equilibrium with tritium in the body fluids, the value obtained for concentration in the urine accurately measures the concentration in body water. The average concentration of tritium in the body fluids over the 5-day exposure period was used to calculate the total dose of beta radiation delivered to the rats.[a] The doses calculated were 314,

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[a] $\text{rep} = \frac{(A)\ (D)\ (E)\ (T)\ (C)}{R}$, where A = activity in μc/ml in body fluids, D = disintegrations/sec/μc (3.7×10^4), E = av. energy of tritium beta particles in ev (6×10^3), T = exposure time in sec (5 days = 4.32×10^5 sec), C = gm of water per gm of tissue (0.75) and R = ev/rep/gm of tissue (5.8125×10^{13}).

362, 423, 455, 511, and 636 rep, respectively, for each of the experimental groups. The amounts of HTO injected, the HTO concentration in the drinking water, the expected HTO concentration in body water, the concentration found in the urine, and the calculated rep delivered are shown in Table 2.

3.4 Measurement of Fe^{59} Uptake

Maximum differences in Fe^{59} uptake by red cells in animals exposed to various doses of radiation have been shown to occur 3 days after the injection of the radio-iron.[6] Therefore, the tracer doses of Fe^{59} were injected 3 days before the end of the exposure period, i.e., 48 hr after the beginning of exposure. The iron was injected as $FeCl_3$ diluted with normal saline so that 1.0 ml contained 0.3 μc of activity. The solution was injected into the surgically exposed jugular sinus under direct observation. Each rat received 0.3 ml (0.09 μc) of Fe^{59}. At the end of the exposure period (5 days) the animals were anesthetized, weighed, and bled by cardiac puncture, using syringes wet with heparin. One milliliter of blood obtained from each rat was placed in a hematocrit tube (capacity of 1 ml) and centrifuged at 2,600 rpm for 30 min. The hematocrit was measured and 1/2 ml of plasma was placed in a small vial for radio-assay. The remaining plasma and the packed red cells were placed in a second vial for a similar assay. The determinations of Fe^{59} in the various samples were made by "gamma counting" in a scintillation counter, using a thallium activated sodium iodide crystal. The counter used was similar to that described in detail by other authors.[8]

TABLE 2

EXPOSURE CONDITIONS AND EFFECT OF H^3 BETA RADIATION ON THE PERCENTAGE OF NORMAL IRON UPTAKE BY THE RED BLOOD CELLS OF RATS

Group	HTO injected per 200 gm, mc	Expected HTO conc. in body water, μc/ml	HTO conc. in drinking water, μc/ml	HTO conc. in urine, μc/ml	Dose, rep	Normal Fe^{59} uptake percent(*)	Standard error of mean
1	72.0	480	640	514	636	4.1	1.4
2	63.4	422	563	413	511	12.6	6.2
3	54.7	365	486	368	455	16.5	8.3
4	47.5	317	422	342	423	19.9	13.2
5	42.5	278	371	293	362	34.5	15.8
6	36.0	240	320	254	314	38.8	11.7

(*) Iron-uptake, control = 59.4 ± 7.13 percent.

Calculations of the amount of Fe^{59} incorporated in red blood cells were performed by the method described by Hennessey and Huff.[9(b)]

4. Results

Increasing doses of total-body gamma radiation from Co^{60} or total-body beta radiation from tritium produced increasingly greater depression of the amount of Fe^{59} incorporated in the red blood cells. The average percentage of normal (control) uptake by rats following various radiation doses is shown in Table 1 (page 8) and Table 2 (page 11).

If the data are plotted on semilog paper as log dose versus percentage of control uptake, a straight line of the type $Y = a + b \log X$ best fits to the experimentally determined points. The data showing the effect of Co^{60} gamma and tritium beta radiation on Fe^{59} uptake by red blood cells are plotted in Fig. 2. The two regression lines (calculated by the least squares method) may be expressed as follows:

Co^{60} radiation, $Y = 333.27773 - 109.41849X$

Tritium radiation, $Y = 336.86176 - 119.52987X$

where Y = percentage of control and X = log dose in r or rep.

It should be emphasized that in the case of Co^{60} radiation each

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(b) $\frac{(A)(B) - (C)(D)}{E} \times 100$ = percent of Fe^{59} in red blood cells

where A = whole blood volume, B = c/min/ml whole blood, C = plasma volume, D = c/min/ml plasma and E = c/min injected.

$\frac{Fe^{59} \text{ in cells (exptl.)}}{Fe^{59} \text{ in cells (control)}} \times 100$ = percentage of normal Fe^{59} uptake.

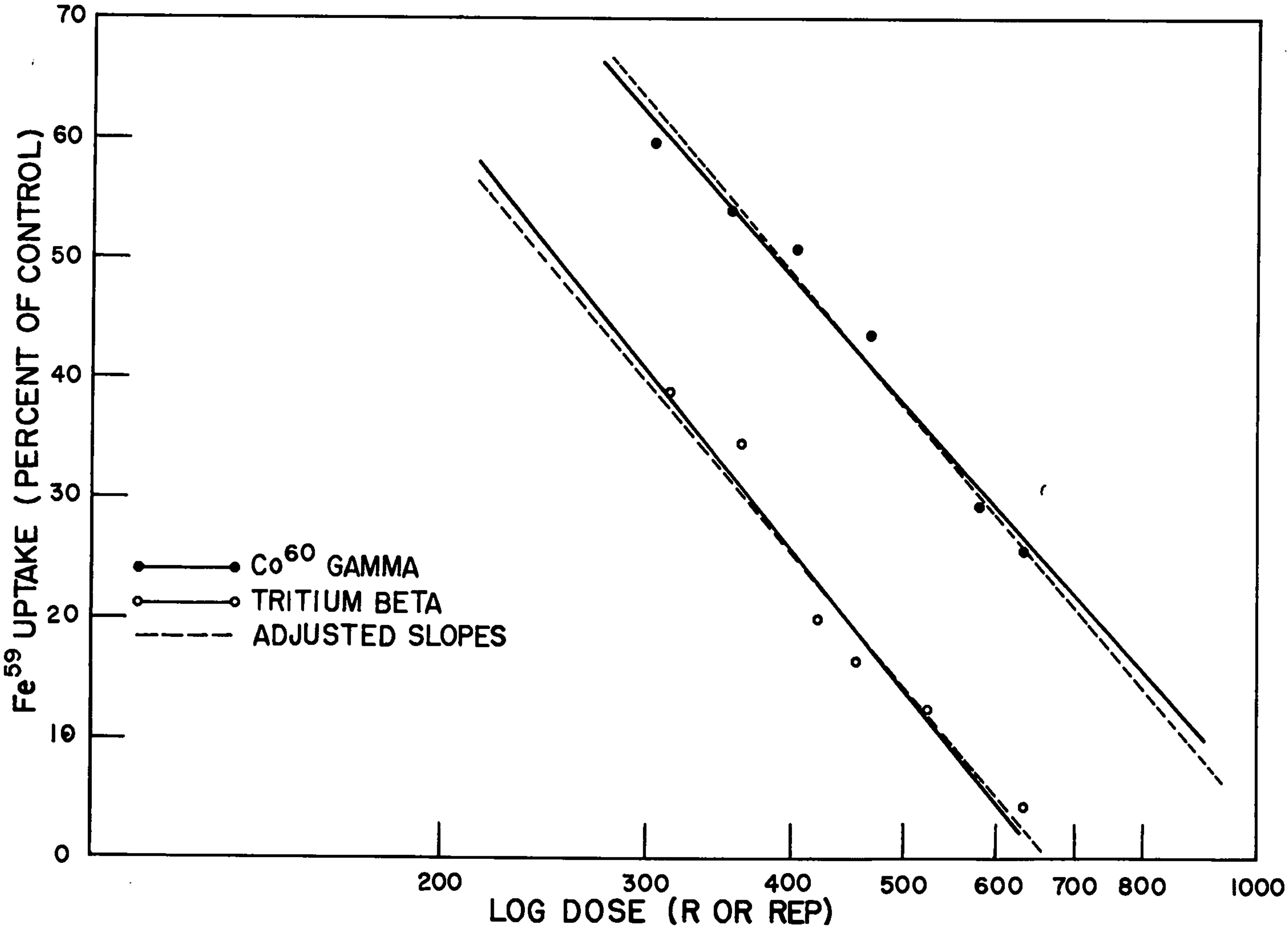

Fig. 2. Percent of normal Fe^{59} uptake by red blood cells of rats as a function of dose of Co^{60} gamma and H^3 beta radiation.

experimentally determined point (Fig. 2) represents the average value for 10 animals. In the case of tritium radiation, each point represents the average value for 12 animals.

The standard errors of the mean values for percentage of normal Fe^{59} uptake following the various radiation dosages are shown in Tables 1 and 2. The relatively large values for these standard errors are believed to result from the algebraic summation of two factors, namely, the degree of radiation damage produced by the radiation and the extent of recovery from this damage. Studies of the effect of acute exposures to X radiation and neutrons showed standard errors about half the size found in the present study. The smaller errors in data from animals exposed to acute doses of radiation were believed due to the fact that only the extent of radiation damage was measured, whereas in the present study the two variables, extent of damage and extent of recovery, were measured. Despite this error in the individual points, the points themselves deviated little from the regression lines and the standard error of the estimate for the regression lines was 5.73436 and 8.55953 for animals exposed to Co^{60} and tritium, respectively.

Determination of the significance of the difference in slope of the two regression lines indicated that there was an 80 percent likelihood that the difference was due to chance. It was concluded, therefore, that there was no significant difference in the slope. The slopes were adjusted to be the same by weighting each by the inverse of its variance and taking the average. New values for the intercept constants a, were then calculated. The adjusted values for the slope and the new values of a gave the following equations for the lines of regression:

Co^{60} radiation, Y = 346.19154 - 11429483X

Tritium radiation, Y = 323.06465 - 114.29483X

where Y and X have the same significance as in the unadjusted equations. Thus at all levels of dosage the relative effect of tritium beta radiation to Co^{60} gamma radiation was constant, as measured by the uptake of Fe^{59} by red blood cells. This relative effectiveness can be calculated from the equation:

$$E = \frac{a_c - a_t}{b}$$

where a_c and a_t are the intercept constants for Co^{60} gamma radiation and H^3 beta radiation, respectively, and b is the common slope constant of the equations for the lines of regression. Substitution in and solution of the above equation gives an RBE of 1.593 ± 0.107 for tritium beta rays in terms of Co^{60} gamma radiation. In other words, on an energy (rep) basis, the beta radiation from tritium was 1.59 times as effective as the gamma radiation from Co^{60} in producing depression of Fe^{59} uptake by the red cells of rats.

Values for RBE are conventionally expressed in terms of 250 KVP X rays. If the RBE of Co^{60} gamma radiation for rats and mice is 1,[10] the RBE of tritium beta radiation in terms of 250 KVP X rays is 1.593 ± 0.107 also.

5. Discussion

The value of 1.59 for the RBE of tritium beta particles is in fair agreement with the previously reported data indicating that the value was slightly greater than 1,[3] and also with unreported data from this laboratory by Worman who found an average of 1.36 when compared with radium gamma radiation using weight decrease of the mouse spleen and thymus. It is interesting that this degree of agreement was found even though Fe^{59} uptake in rats was used as an end point of effect in the present study, whereas in previous studies lethality and organ weight decrease in mice were used.

The value is also in good agreement with the RBE value of 1.4 reported for Na^{24} beta radiation.[4] In that case, lethality in rats was used as the end point for measuring effect and Na^{24} was used as an internal emitter. The close agreement between the RBE for tritium beta radiation found in the present study and the RBE for Na^{24} raises a question as to whether any good correlation exists between specific ionization and RBE as has been suggested.[11] Calculations of the specific ionization for tritium and Na^{24} indicate that the average specific ionization of tritium beta particles is about 30 times that of Na^{24} beta particles. Inasmuch as the RBE's of the two beta radiations are not significantly different it may be concluded that, at least in the systems studies, there is no correlation between RBE and the specific ionization.

Since there appears to be no significant difference between the RBE of the beta rays from tritium and from Na^{24}, even though there is a thirty-fold difference in specific ionization, it seems doubtful that there is any difference between the RBE of tritium beta rays and alpha particles from such substances as plutonium, where there is only a ten-fold difference in specific ionization. This reasoning is supported by the report of Hollcroft and Lorenz[12] in which the RBE of radon alpha particles was found to be 1.42. If subsequent investigations support this line of reasoning it may be possible to revise downward the value of 20 for the RBE of alpha particles set by the Chalk River Conference.[1]

Taking the maximum amount of tritium permissible in the body as 10 mc and the RBE of the tritium beta as 1.5, the radiation dose delivered by the tolerance amount of tritium would be 0.45 rem/wk.[c] On this basis, the amount of tritium required to deliver 0.3 rem/wk to a 70 kg man would be 7 mc, which probably does not differ sufficiently from the recommended value of 10 mc to have any practical significance.

6. Summary and Conclusions

A comparison of the effects of exposure to Co^{60} gamma radiation and tritium beta radiation on the bone marrow of rats was made by comparison of the amounts of intravenously injected Fe^{59} incorporated in the red blood cells. The percentage of normal Fe^{59} uptake varied inversely with the log of the dose. Analysis of the statistical parameters

[c] One rem is defined as that dose of ionizing radiation which produces a relevant biological effect equal to that produced by 1 r of high voltage X radiation, other exposure conditions being equal.

of the data showed that the beta radiation of tritium was 1.59 times as effective as the gamma radiation of Co^{60} in producing depression of radio-iron uptake. Comparison of the RBE so obtained with the RBE of other radiations with specific ionizations varying by an order of magnitude suggest that there is no direct relationship between specific ionization and RBE.

It was felt that the RBE obtained for tritium beta particles was not sufficiently different from 1 to warrant reconsideration of the 10 mc maximum permissible amount of tritium recommended for man.

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