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BIOLOGICAL EFFECTIVENESS OF THERMAL NEUTRONS IN PRODUCING TESTICULAR ATROPHY IN MICE

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ABSTRACT

Using testicular atrophy in mice 4 weeks post-exposure as the biological indicator of radiation effect, a comparison was made of the relative effectiveness of thermal column and 250 KVP X radiation. By assuming an RBE of unity for the known gamma contaminants associated with exposure to the thermal neutron flux of the Los Alamos Homogeneous Reactor, it was found that $\sim 8 \times 10^9$ thermal n/cm^2 produced an effect equivalent to 1 r of 250 KVP X rays. Depending on assumptions regarding the depth of the testes with regard to the region of peak neutron collision density, calculation of the RBE for thermal neutrons gave values of from 1.2 to 2.5, with 1.3 being the best value obtainable from the present study.

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1. Introduction

In all considerations of permissible human exposure to various types of ionizing radiations the relative biological effectiveness (RBE) of the particular radiation in terms of X or gamma rays must be taken into account. The problem of establishing a value for the RBE is complicated by the difficulty of measuring physical dose of the radiations in energy units (ergs or rep) and by the fact that effectiveness may vary, depending on the particular tissue or response studied and whether acute or chronic exposures are employed. For the latter reason it is obvious that the value for the RBE of any particular type of radiation should be based on data obtained in a wide variety of test systems following both acute and chronic exposure.

Information has been obtained in this laboratory on the RBE of thermal neutrons in producing acute lethality in mice[1] and rats,[2] atrophy of the spleen and thymus in mice,[3] depression of mitotic activity in mice,[4] depression of Fe^{59} uptake by the red blood cells of rats,[5] incidence of lens opacities in mice,[6] and incidence of tumors and shortening of the life span in mice.[7] All of the above studies have been performed on animals that had veen given a single exposure to neutrons. Additional information is being obtained regarding the effect of multiple thermal neutron exposure on the life span of mice and on the incidence of lens opacities and tumors.[7] In all the systems so far studied, with the exception of the lens of the eye, the RBE for thermal neutrons given in a single exposure has been found to lie roughly between 1.0 and 2.0.

The germinal epithelium of the gonads of animals is known to be radio-sensitive and the question is often raised as to whether or not the gonads may be particularly sensitive to neutron irradiation. Neary et al.,[8] in a preliminary report, have indicated that the mouse testis is extremely sensitive to chronic irradiation with fast neutrons. Little or no information exists as to the relative effectiveness of thermal neutrons on this organ.

The present study was undertaken to determine the relative biological effectiveness of thermal neutrons in producing testicular atrophy in mice following a single acute exposure. It is anticipated that similar studies on the effects of chronic exposure may be performed in the future.

2. Methods

2.1 Testicular Atrophy as a Biological Indicator

Eschenbrenner and Miller[9] have shown that there is a quantitative relationship between the degree of testicular atrophy in mice and the dose of X rays delivered. This relationship has been used in the present study to obtain a quantitative measurement of the RBE of neutrons on the testis. Preliminary experiments showed that maximum atrophy was obtained at 4 weeks,

and that when the mean testicular weight (in milligrams per gram of body weight) of the irradiated mice was plotted as percentage of the mean testicular weight of the control mice the relationship to dose could be expressed by a formula of the type

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

where Y = per cent of control testicular weight, X = dose of radiation, a = intercept constant, and b = slope constant.

The above relationship was found to hold over a dosage range of 50 to 300 r of X rays and over a similar range for thermal neutrons when the neutron dosage was expressed in rem.*

In the present study male mice were exposed to single acute doses of thermal column or X radiation. Four weeks after exposure they were sacrificed with ether, and the body weights and the wet weights of the testes were determined.

2.2 Randomization and Handling of Animals

A total of 390 adult male CF_1 mice 4 to 5 months of age were used. Prior to irradiation the mice were randomized by placing them in a large rectangular open-top box from which they were randomly reassigned to cages. Prior to and following radiation exposure the animals were maintained 30 to a cage and given water and Purina laboratory chow ad libitum.

One group of 210 mice was exposed to graded doses of thermal column radiation. A second group of 150 was exposed to graded doses of X radiation, and a third group of 30 was set aside to serve as unirradiated controls. Because of the fighting nature of adult male mice of this strain, many of the mice succumbed to the combined effects of fighting and irradiation. Table 1 lists the number of animals successfully maintained to the end of the 4 weeks period and also lists the radiation dosages employed.

2.3 X-Ray Exposure

The X-ray exposure techniques were described in detail in a previous report.[1] Briefly, the exposure conditions were as follows:

(1)	Type of machine:	Picker industrial model
(2)	Peak voltage:	250 kv
(3)	Inherent filtration:	3 mm Al
(4)	Added filtration:	0.33 mm Cu + 0.83 mm Al

*One rem is that dose of any 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.

(5) Dose rate (free air): 59.6 r/min

(6) HVL in Cu: 2.0 mm

(7) Target-specimen distance: 55 cm

(8) Filament current: 15 ma

Measurements of radiation intensity were made in air with 100 r Victoreen thimble chambers, and appropriate correction factors were applied.

The mice were exposed 30 at a time in a shallow Lucite cage curved on a radius of 55 cm to minimize differences in radiation intensity between the center and periphery of the cage.

2.4 Thermal Column Exposure

The details of exposure of mice to thermal column radiation have been described in a previous report, along with the characteristics and description of the reactor.[1] Briefly, the exposure conditions were these. Mice were exposed 15 at a time inside the north tunnel of the Los Alamos Homogeneous Reactor. The reactor was operated at 5 to 25 kw, depending on the total dose of radiation desired. In cases where radiation doses were low the reactor was operated at lower power levels in order to increase the irradiation times. The increased times facilitated greater accuracy of dose measurements. Since the thermal column output increases directly with power level, all the times given in Table 1 have been converted, for consistency of presentation, to times that would have been required at an operating level of 25 kw.

With the reactor operating at 25 kw it has been shown that 1 sec of exposure delivered 1.42×10^{10} thermal n/cm^2 plus 0.41 r of inherent gamma having an energy of $\sim$4 Mev. In addition to the inherent gamma rays there was a second gamma contaminant of lower energy caused by activation of the mice themselves so that they served as gamma sources. This second contaminant contributed 0.15 r/sec.[1]

3. Results

The data on testicular weight (in milligrams per gram of body weight) 4 weeks after exposure to thermal column or X radiation are summarized in Table 1. For convenience in statistical treatment the radiation dosages are expressed in seconds of exposure. Since X rays were delivered at the rate of 1 r/sec it is obvious that the values for exposure times in seconds are equal to the total roentgen dosages. From this table it can be seen that over the dosage ranges employed the testicular weights were smaller with increasing doses of radiation.

Figure 1 shows a plot of testicular weight (as percentage of control weight) against the log of the time of radiation exposure. The regression line for a linear plot is of the type:

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

The regression lines were calculated by the least squares method without weighting of the various points. The following formulae were obtained:

X ray: $Y = 162.40 - 44.37 \log X$

Thermal column: $Y = 147.61 - 45.16 \log X$

To express the effectiveness of thermal column radiation in terms of X radiation over the entire range of dosages used it is necessary to substitute into the formula

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

where E = relative effectiveness, a_x = intercept constant for X ray, a_t = intercept constant for thermal column exposure, and b = common slope constant for the regression lines.

Substitution into this formula can be done only if the two regression lines have a common slope (b). It was obvious that the two values for b of 44.37 and 45.16 for X rays and thermal column exposure, respectively, were not significantly different. An arithmetic average of the two gave a value for 44.77 for the common slope. Recalculation of the regression lines using this value for b gave:

X ray: $Y = 163.24 - 44.77 \log X$

Thermal column exposure: $Y = 146.82 - 44.77 \log X$

Substitution into the above formula for the effectiveness of one radiation in terms of another showed that 1 sec thermal column exposure was equivalent to 2.3 sec X-ray exposure. Since the X rays were delivered at 1 r/sec it is apparent that 1 sec of thermal column exposure delivered 2.3 rem under the conditions of the experiment assuming equivalence of r and rem for 250 KVP X rays.

To determine the effectiveness of thermal neutrons per se in producing testicular atrophy it is necessary to subtract the effect contributed by the gamma contaminants present in the thermal column radiation. Such subtractions of effect have been made routinely in previous studies[1, 3, 4, 6] and the validity of the practice is supported by experimental data.[10] These contaminants contributed a total of 0.56 r/sec. In order to subtract their effect from the total it is necessary to assign a value for RBE. The only study in which the effectiveness of the gamma contaminant on a tissue lying close to the surface of the mouse was measured gave a value for RBE of 1.0. This value was found to hold for the effect on the lens of the eye. Since the testes are located superficially it seems reasonable to assume that the same value for RBE applies to them. If this assumption is made, then the total rem contributed by thermal neutrons alone may be calculated as follows:

Mixed thermal column radiation:	2.30 rem/sec
Gamma contaminants:	-0.56
Thermal neutrons:	1.74 rem/sec.

Neutrons were delivered at the rate of 1.42×10^{10} n/cm^2/sec. Since this number of neutrons delivered 1.74 rem it may be concluded that the value for 1 rem of thermal neutrons in producing testicular atrophy in mice is approximately 8×10^9 n/cm^2.

To determine the RBE of the neutrons it is necessary to know the number of rep delivered. Assuming the mouse to be a thin foil, Brennan et al.[1] calculated that under conditions identical to those used in the present study the thermal neutrons delivered 0.61 rep/sec. Brennan's calculation, however, assumes that the mouse has a 3% nitrogen content. More recent studies indicate that the proper value is closer to 4%. Recalculation of Brennan's data with the 4% value gave a figure of 0.70 rep/sec. If this value for rep is used, then the RBE of thermal neutrons is

$$\frac{1.74 \text{ rem/sec}}{0.70 \text{ rep/sec}} = 2.5$$

The thin foil assumption, however, probably does not apply in the present case since the peak neutron collision density according to Snyder[11] lies ~0.32 cm below the surface. Since the testes of mice lie in this region of depth from the surface the increased neutron collision density must be considered. Snyder's calculations are based on energy absorption in a semi-infinite homogeneous half space where neutrons would not be lost except at the surface of the medium and gamma ray energy would not be lost through leakage. This situation certainly does not hold for the mouse, particularly with respect to absorption of gamma ray energy. In addition, it is unlikely that no neutrons would be lost in the case of the mouse. If neutrons were lost, the energy deposited by the N(n, p)C reaction at the point of peak collision density would be less for the mouse than for Snyder's theoretical semi-infinite homogeneous half space. No calculations are available for the mouse so that for the present it is necessary to assume that the conditions in the two systems are similar. Using this assumption and the further assumption that the absorption of gamma ray energy at the point of maximum neutron collision density in the mouse is, on the average, the same as the average absorption in the rest of the mouse, the number of rep delivered may be recalculated. At ~0.32 cm below the surface with an incident flux of 1.42×10^{10} thermal n/cm^2/sec the following rep values are found:

N(n, p)C reaction =	1.24 rep/sec
H(n, γ)D reaction =	0.26 rep/sec
Total =	1.50 rep/sec

The remaining reactions between neutrons and chemical constituents of the mouse, such as the $B(n, \alpha)Li$ reaction, contribute so little energy as to be negligible. Since it was found that the thermal neutrons contributed 1.74 rem/sec in producing testicular atrophy, the RBE (assuming the testes to lie at the point of peak neutron collision density) equals

$$\frac{1.74 \text{ rem/sec}}{1.50 \text{ rep/sec}} = \sim 1.2$$

The obvious error in the above calculations is in assuming that the entire testis lies at the point of peak neutron collision density. A more reasonable assumption would be that the neutron collision density in the testis lies somewhere between the value for peak density and the value for the density at the surface. In this case the rep values based on an average of the peak and surface collision densities become

$N(n, p)C$ reaction:	1.09 rep/sec
$H(n, \gamma)D$ reaction:	0.26 rep/sec
	1.35 rep/sec

and the RBE becomes

$$\frac{1.74 \text{ rem/sec}}{1.35 \text{ rep/sec}} = 1.3$$

4. Discussion

The degree of testicular atrophy produced in mice 4 weeks after exposure either to X rays or to thermal column radiation was found to increase linearly with the log of the radiation dose. Since the slopes for the regression lines for response as a function of radiation dose were parallel it was possible to compare the effectiveness of the two dissimilar radiations over the entire range of dosages employed. By assuming an RBE of 1.0 for the know gamma contaminants associated with exposure to the thermal column flux and subtracting their effects from the total it was found that $\sim 8 \times 10^9$ thermal n/cm^2 produced an effect equivalent to 1 r of 250 KVP X rays.

To calculate the RBE of the thermal neutrons in producing testicular atrophy it was necessary to calculate the rep dose delivered by the neutrons. If it is assumed that the mouse is a thin foil and that the rep dose to the testis is the same as the average rep dose to the rest of the body, then the RBE for neutrons in producing testicular atrophy is 2.5. Since the testes lie superficially, however, the thin foil assumption probably does not apply and some adjustment in the calculated rep value must be made to allow for the fact that at least a portion of the testis lies in the range of the peak

neutron collision density. The calculations of Snyder[11] for energy deposition at this point do not strictly apply since his calculations are for a semi-infinite homogeneous half space where no neutrons are lost through leakage except at the surface and no gamma ray energy is lost from the tissue. Snyder's data for energy deposition from the N(n, p)C reaction at the point of peak collision density probably very nearly apply for the mouse although neutron leakage would probably lower the value slightly. The values for gamma ray absorption from the $H(n,\gamma)D$ reaction certainly do not apply to the mouse. It would be more reasonable to assume that the gamma ray energy deposition is similar to the average for the rest of the mouse (based on a thin foil assumption). When these values are used to calculate the RBE of thermal neutrons in producing testicular atrophy the value becomes ~ 1.2.

It is obvious that the entire testis of the mouse does not lie at the point of peak collision density. Perhaps a better value for rep would be obtained by assuming an average of the energy deposition at the surface and at the point of peak collision density. In this case the RBE becomes ~ 1.3.

From the data presented it appears that the RBE of neutrons in producing testicular atrophy in mice lies between 1.2 and 2.5, with 1.3 being perhaps the best value presently obtainable. This value is undoubtedly slightly low since the assumption that there is no leakage of neutrons tends to maximize the rep values calculated and thereby decrease the value for RBE.

The value for RBE of 1.3 compares favorably with the value 1.5* calculated from the data of Brennan et al.[1] using 30-day lethality as the biological response. It also compares favorably with the respective values of 1.65, 1.7, and 1.14 obtained for the RBE of thermal neutrons using splenic and thymic atrophy[3] and depression of the mitotic index in mice,[4] and depression of Fe^{59} uptake in rats.[5] This close agreement with the RBE values found in other test systems strongly suggests that there is not specific sensitivity of the mouse testis to thermal neutrons once the superficial anatomical location is taken into account and allowance thereby made for the increased radiation dose delivered. From the present study no conclusions can be reached as to the effectiveness of the neutrons in producing sterility, but inasmuch as no specific effect in producing atrophy was obtained it seems unlikely that any specific effect on fertility would be observed.

*Brennan et al.[1] originally reported an RBE of 1.7. This calculation was based on the assumption that a mouse contains 3% nitrogen. In the present report a value of 4% is assumed. Recalculation of Brennan's data, using 4% nitrogen, gives an RBE of 1.5.

5. Summary and Conclusions

Using testicular atrophy in mice 4 weeks post radiation as the biological indicator of effect, a comparison was made of the relative effectiveness of thermal column and 250 KVP X radiation. By assuming an RBE of 1.0 for the known gamma contaminants associated with exposure to the thermal neutron flux of the thermal column it was found that $\sim 8 \times 10^9$ thermal n/cm^2 produced an effect equivalent to 1 r of 250 KVP X rays. When the dose of thermal neutrons was calculated in rep, assuming the mouse to be a thin foil and that the dose to the testes was the same as to the entire body, the RBE of thermal neutrons was 2.5. When the RBE was calculated on the assumption that the testes lie at the depth of peak neutron collision density, a value of ~ 1.2 was obtained. Assuming an average of the surface and peak neutron collision density, the RBE of thermal neutrons was found to be ~ 1.3.

From the present study it was concluded that the RBE of thermal neutrons for producing testicular atrophy in mice lies between 1.2 and 2.5 with ~ 1.3, although probably too low, being perhaps the best value presently obtainable.

The value of 1.3 for the RBE of thermal neutrons in producing testicular atrophy in mice compares favorably with values of 1.5 for 30-day lethality, 1.65 for spleen and thymus weight decrease, 1.7 for depression of mitotic activity of the skin of the mouse ear, and 1.14 for the uptake of Fe^{59} by the red blood cells of rats.

No definite conclusion could be drawn as to the effectiveness of thermal neutrons in producing sterility, but inasmuch as no specific effect in producing atrophy was obtained it seems unlikely that any specific effect on fertility would be observed.

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TABLE 1

EFFECT OF THERMAL COLUMN AND X RADIATION ON THE TESTICULAR WEIGHT OF MICE 4 WEEKS AFTER EXPOSURE

Type of Radiation	Dose (r or sec)	No. Mice	Mean Testicular Wt (mg/gm body wt)	Per Cent of Control Wt
Thermal Column	33.6 sec	22	5.01 ± 0.108	79.2
Thermal Column	73 sec	40	3.97 ± 0.072	62.8
Thermal Column	109.5 sec	7	3.48 ± 0.123	55.0
Thermal Column	146 sec	37	3.17 ± 0.070	50.1
Thermal Column	218 sec	27	2.68 ± 0.107	42.4
X Ray	50 r	21	5.49 ± 0.141	86.8
X Ray	100 r	18	4.66 ± 0.147	73.7
X Ray	150 r	18	4.16 ± 0.198	65.8
X Ray	200 r	21	3.87 ± 0.093	61.2
X Ray	300 r	11	3.28 ± 0.140	51.8
None (Controls)	---	19	6.32 ± 0.152	100

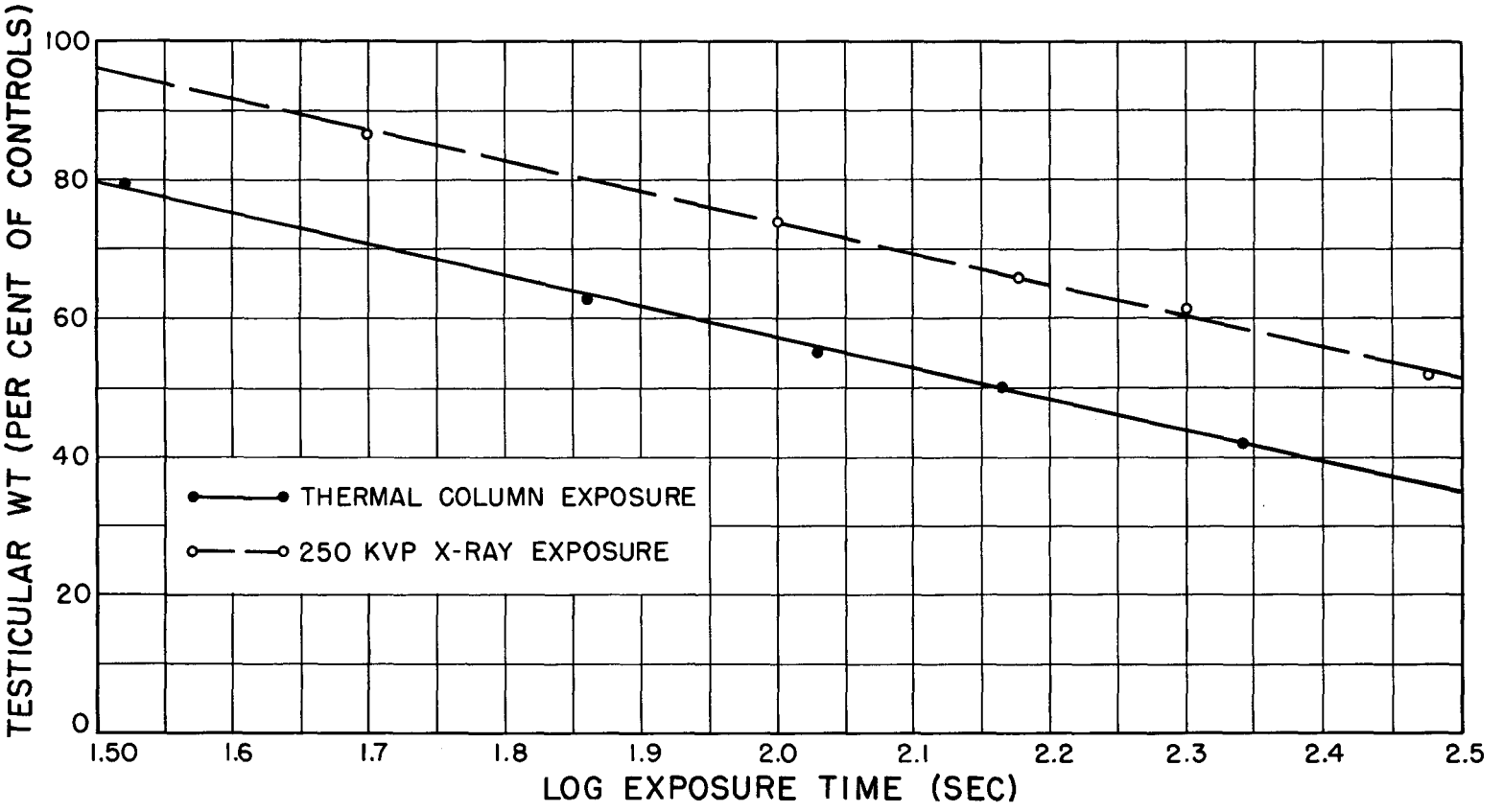

Fig. 1 Effect of thermal column and X radiation on testicular atrophy of mice 4 weeks after exposure.