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RADIATION RESEARCH **51**, 381–390 (1972)

Dose-Dependence of Fast Neutron RBE for Lens Opacification in Mice[1]

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BATEMAN, J. L., ROSSI, H. H., KELLERER, A. M., ROBINSON, C. V., AND BOND, V. P. Dose-Dependence of Fast Neutron RBE for Lens Opacification in Mice. *Radiat. Res.* **51**, 381–390 (1972).

Opacification of the optic lens in mice has been evaluated after exposure to the following radiations and doses: 250 kVp x-rays, 4.5–1,000 rads; 14 MeV neutrons, 0.5–60 rads; 1.8 MeV neutrons, 1–36 rads; and 0.43 MeV neutrons, 0.02–104 rads.

Highly refined techniques of serial *in vivo* lens examination and scoring of minute defects (opacities) were employed for this very radiosensitive system. Data analysis was based on the relative accumulation of opacities in irradiated compared to untreated lenses. (Both revealed the same degenerative process.)

A nonparametric analysis of the observation discloses that the RBE is inversely proportional to the square root of the neutron dose and that it can reach values in excess of 100. The findings are in accord with the theory of dual radiation action.

INTRODUCTION

The mammalian optic lens, long of interest for radiation hazard considerations in man, has also served as an assay system of radiobiological value for many investi-

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gators. The inspections for accumulated visibly defective lens fibers, descendant from the relatively hypoxic proliferative lens epithelium are serial and nondestructive. These features combine to create a potent investigative tool, despite limitations due to the necessity to employ an arbitrary, but reproducible, observer-based scoring scale. This difficulty is largely eliminated if the relative biological effectiveness (RBE) is analyzed.

This series of studies was stimulated by the development of a monoenergetic fast neutron technique (*1*) which was first employed at this laboratory for the study of early effects in mice (*2*, *3*). The available discrete energies of neutrons provide, along with comparison with x-rays or ^{60}Co gamma rays, a broad selection of radiation quality which is of evident value toward the clarification of basic radiation mechanisms.

Some of the experiments described here have been dealt with in part in previous papers (*4*, *5*). Radiation doses of intermediate magnitude were involved, and lens opacification in the anterior axial (epithelial) "A" region and in the posterior subcapsular (fiber) "V" region were both termed "radio-induced" despite the evidence of changes with age in the controls which were indistinguishable from those appearing earlier in the irradiated lens.

The present paper assembles the data from the earlier studies and two more recent studies which employed doses which evoke no detectable effects in most other animal and plant systems. The low-dose region was of particular interest because the results in the first two lens experiments were compatible with the generally held view that fast-neutron RBE values rise with decreasing dose.

The present studies are consistent with ionizing radiation acting by increasing the rate of a natural aging process, rather than by creating an abnormal *de novo* situation.

MATERIALS AND METHODS

Exposures to x-rays were performed with a 250 kVp G.E. Maxitron therapy unit operating at 30 mA. In the various experiments the HVL ranged from 1.25 to 2.5 mm Cu and the dose rate from 17 to 115 rads/minute[6]. Unit density material was employed in thicknesses of several mm for buildup and about 10 cm for backscatter.

Exposures to neutrons of 0.43 and 1.8 MeV were carried out as previously described (*4*).

Exposures to 14 MeV neutrons utilized a Van de Graaff generator to produce the

[6] The x-ray dose rates were substantially higher for Expts G and H than for Expt D. The difference is not considered to be important to the effects discussed in this paper. In a separate study (*17*) no difference was found between 2 and 100 rads per minute for lens opacification resulting from exposure to 250 kVp x-rays made under hypoxic conditions. The sensitivity (opacification) of the lens in these latter exposures resembled that of previous experiments with exposures made in room air (see below).

$^3H(d,n)$ ^4He reaction. Mice were located on an eight-station circle, with body axes pointed at the target, at a 75° angle to the forward beam axis. The mouse noses were pointed towards the target, with the eyes at 10.3 cm distance from the center of the target. The dose was measured by means of a specially constructed detector which is a phantom in the shape of a mouse containing a small toroidal ionization chamber in the general location of the eyes. It has been described previously (*6*).

Table I gives the protocols for the four experiments which yielded the data analyzed in this publication. Except for a few double groups each experimental

TABLE I

PROTOCOLS OF EXPERIMENT

Expt	*Radiation type and absorbed dose in rads*				*Total number of examinations*[a]
	250 kV *x-rays*	14 MeV *neutrons*	1.8 MeV *neutrons*	0.43 MeV *neutrons*	
	0 (*Controls*)				
	4.5	0.55		0.022	
M	12.0	1.65		0.106	13
	30.0	4.83		0.531	
	0 (*Controls*)				
	6.0	1.0		0.54	
	12.0	4.5		1.08	
H	24.0	14.8		2.08	17
	48.0	30.5		4.10	
				6.05	
	0 (*Controls)				
	50*		1.2*	1.1*	
D	150		6.1	5.3	6
	350		18.2	15.9	
			36.4	31.8	
	0 (*Controls*)				
	70			7.2	
	160			16.2	
G	260			27.0	14
	400			41.3	
	600			61.2	
				104	

[a] Each examination involves typically 40 lenses in each group except for 80 in groups marked by an asterisk (*).

group involved about 24 mice in which at least 40 lenses were scored at each examination. The excess number of animals permitted sustained scoring of 40 lenses despite losses by death of some of the animals.

BNL-Stoner strain (white Swiss) female mice were employed in these investigations. The animals averaged 13 weeks of age at the time of irradiation in some of the experiments; in Expts H and G the average age was six weeks. Supporting studies indicate that radiosensitivity is somewhat increased at an earlier age. Although such a difference would be unacceptable in dose-effect studies, it should have far less (if any) effect on RBE studies. In fact the higher-dose results obtained in the H and G experiments agree with those of the other experiments.

The mice were maintained on Purina Chow pellets and water ad lib. Temperature and humidity were controlled and there were ten mice to a cage. Serial examinations with a 160 power Bausch & Lomb slit-lamp microscope were begun at four weeks after exposure, with an initial interval of one week between examinations, gradually lengthening to three weeks by one year postirradiation. Mice were examined in the conscious state, after dilation of their pupils by 1 % homatropine. Exposure-group assignment of mice was not known to the observer until all animals had been scored. Mice were held rigidly in a fixture permitting rapid and accurate positioning for examination.

Visible opacities were scored as to size and recorded separately for the anterior "A" and posterior "V" regions of the lens. The former appears to be a hyperplastic lesion which begins with a "piling up" of cells in the central portion of the lens epithelium and proceeds to intrusion of a portion of this mass among packed mature lens fibers. Actual opacification begins only after the latter event. Opacities in the "V" region begin as scattered discrete tiny defects, probably occurring in the posterior distal portion of lens fibers. It is felt (*7*, *8*) that these develop from lens cells derived from abnormal precursor cells resident in the germinal zone of the lens epithelium. "V" opacification becomes evident more quickly postirradiation and progresses at a faster rate than "A" opacification. The data analyzed here were for "V" opacities only.

EFFECT SCALE AND DATA ANALYSIS

The scale of visual estimate of opacity level has been previously described (*4*) and, although based on individual judgement, it has been found to be highly reproducible. It utilizes 15 progressively larger divisions instead of the three or four arithmetically progressive divisions conventionally used to cover the range from 0 to 100 % opacity (*6*, *9*, *10*). The small intervals toward the lower end of this visual scale appear justified on the basis of sensitivity and reproducibility tests.

Two examples of group scores are given in Table II. The groups selected are from Expt M and are controls and subjects exposed to 22 mrad of 0.43 MeV neutrons. In each group 40 lenses were examined at 13 different times. The latter are

TABLE II

FREQUENCIES OF LENS OPACITY SCORES IN TWO GROUPS OF EXPERIMENT M

Age (weeks) \ Score	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Controls															
21	40														
23	39	1													
25	40														
27	36	4													
29	35	2	3												
31	31	6	2	1											
34	29	8	1		1		1								
37	11	19	6	1	1		2								
41	18	13	5	2				1	1						
44	18	14	1	1	2	1		2	1						
48	23	9	2		2			1	3						
52	12	14	3	3	2	2	1	1	1	1					
56	1	20	10			4	2	1	1	1					
0.43 MeV *neutrons* 0.022 *rad at* 18 *weeks of age*															
21	38	2													
23	38	2													
25	33	5	1	1											
27	30	6	1	1	2										
29	22	11	3	2	1		1								
31	22	12	4	1			1								
34	17	19	3					1							
37	17	16	5		1			1							
41	14	20		1	1	3		1							
44	13	23	1			1		2							
48	3	23	5	3	2		1	2	1						
52	3	15	9	5		3	2	1	1	1					
56		18	10	1	4		1	4	1	1					

given as the age of the animals in weeks; irradiation having taken place at the age of 18 weeks. Although the examples given in Table II represent a total of more than 1,000 individual eye examinations, they cover only about 5% of the total which was nearly 20,000.

At least two methods of data analysis have been used in previous lens opacification experiments including score averaging (9–11) and a dichotomized scale-independent method (*6*). We have considered other more elaborate procedures of which two (Robinson, in preparation, and Kellerer, in preparation) will be reported separately. The method of Kellerer is used for the present analysis. The principal feature of this approach is the elimination of the necessity to construct dose-effect curves before the RBE-dose relation is derived. Instead each group of mice exposed to an x-ray dose D_x is directly compared to each group of mice exposed to a neutron dose D_n. If the effect of D_x is found to be significantly higher than that of D_n, the RBE must be less than D_x/D_n. Conversely, if the effect of D_x is found to be significantly less than that of D_n, the RBE must be greater than D_x/D_n. In this way one excludes certain ranges of RBE values at each neutron dose, and as the result of all comparisons one obtains a confidence region for the RBE-dose relation.

This makes the analysis independent of the arbitrary choice of the scale of effect. Scale independence is also a requirement for the statistical test used in the comparison of x-ray and neutron doses. For this reason a parameter free rank-order test, namely the Mann–Whitney test, has been used. All the lenses belonging to the two groups which are compared are arranged in a common rank order according to their score at a certain time after irradiation. Application of the Mann–Whitney test to this rank order shows whether the effect level in the two groups is significantly different.

No significant differences in the RBE-dose relations have been found when the observations, at different times after irradiation, were analyzed. But the confidence region is most narrow for the latest observations.

The results of the analysis by this method are shown for each of the three neutron energies in Figs. 1–3. These results represent averages over the values obtained when the test was applied to the last three observations in each experiment The solid bars indicate ranges of RBE which are excluded according to the outcome of the individual comparisons. Broad solid bars indicate statistical significance exceeding 99%, light solid bars indicate significance exceeding 95%. Arrows stand for differences in effect level which are not statistically significant. The curves represent the shape of the dose-RBE dependence as postulated by the theory of dual radiation action (*12*) and they have been fitted as explained below. Figure 4 shows all three lines for comparison.

DISCUSSION

It is evident from Fig. 4 that the RBE varies far more with dose than with neutron energy. There is at least a 50-fold variation in the RBE of 0.43 MeV neutrons in the dose range investigated. The RBE for neutrons becomes much more than 10 at low doses and at the energy of 0.43 MeV it appears to exceed 100 at 22 mrads. Such RBE values are far larger than those hitherto reported for any mammalian system An obvious reason for the exceptionally high RBE is that it was possible in our study to accurately assess the effects of doses that are exceptionally low.

Since among all the radiations employed in these studies 0.43 MeV neutrons are

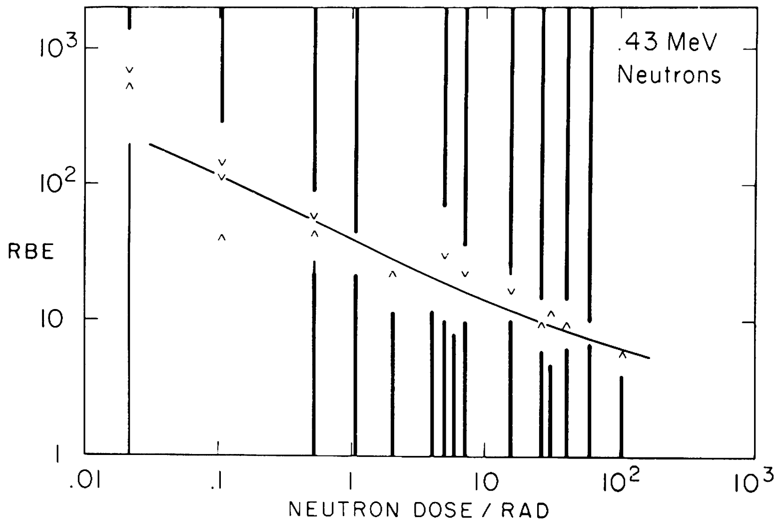

FIG. 1. RBE of 0.43 MeV neutrons relative to x-rays for the induction of lens opacification in the mouse as a function of neutron dose.

The solid bars indicate the ranges of RBE values which, according to the comparison of x-ray and neutron doses are excluded. Broad bars: significance exceeding 99%; light bars: significance exceeding 95%; arrows: nonsignificant differences. The solid line corresponds to a formula given by Kellerer and Rossi (1971).

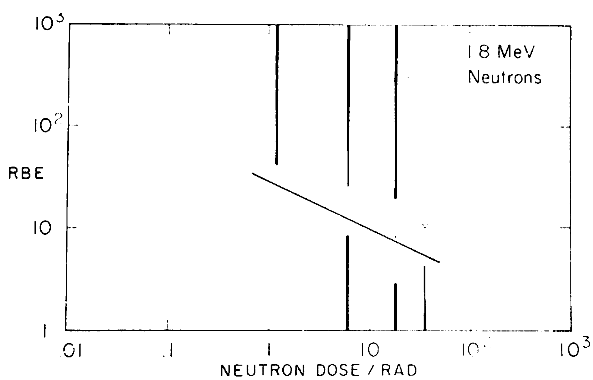

FIG. 2. RBE of 1.8 MeV neutrons relative to x-rays for the induction of lens opacification in the mouse as a function of neutron dose. The representation is analogous to that in Fig. 1.

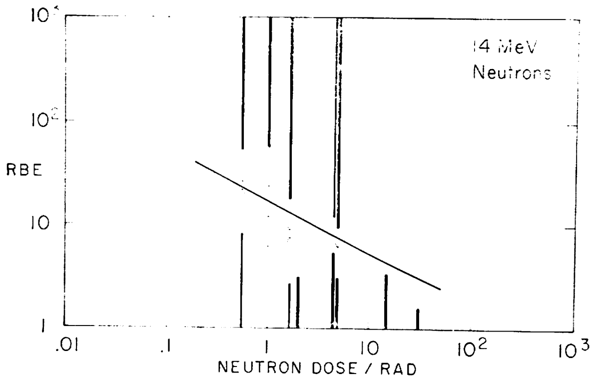

FIG. 3. RBE of 14 MeV neutrons relative to x-rays for the induction of lens opacification in the mouse as a function of neutron dose. The representation is analogous to that in Fig. 1.

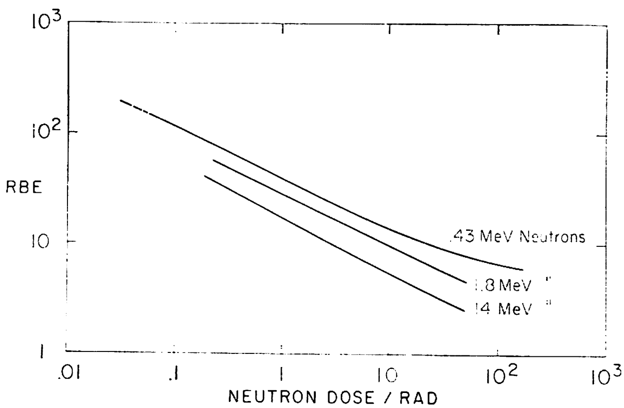

FIG. 4. Comparison of the three dose-RBE relations in Figs. 1–3.

the most potent, they were the subject of especial attention. Their effects were studied over an unusually (and perhaps uniquely) wide 5000-fold variation of dose. It should be noted that at the lowest dose (22 mrad) opacification was found to be greater than in controls with a significance that exceeds 99.9% (see Table II).

The dependence of RBE on dose is the point of departure of the theory of dual-radiation action (*12*). In fact the early development of the theory was in part motivated by the discovery that a portion of the linear section of the curve in Fig. 1 has a slope of $-\frac{1}{2}$ (*13*). According to this theory, elementary lesions responsible for a variety of effects on higher organisms are produced with a yield, ϵ, that is proportional to the square of specific energy, z, (*14*) in regions having a diameter of the order of 1 μm. An equivalent formulation is given by:

$$\epsilon(D) = k(\bar{z}_D D + D^2) \tag{1}$$

where k is a constant, $\bar{z}_D$ the dose average of increments of specific energy (*15*) and D the absorbed dose. Using the subscripts n and x to denote the neutron and x-ray quantities, setting the RBE equal to D_x/D_n for equal ϵ and assuming equal k, one obtains

$$\text{RBE} = \frac{2(\bar{z}_{D,n} + D_n)}{\bar{z}_{D,x} + \sqrt{\bar{z}_{D,x}^2 + 4(\bar{z}_{D,n} + D_n)D_n}} \tag{2}$$

For large and small values of D_n this expression tends to 1 and $(\bar{z}_{D,n}/\bar{z}_{D,x})$ respectively. In the intermediate range of doses the RBE is proportional to $\sqrt{1/D_n}$ if $\bar{z}_{D,n} \gg \bar{z}_{D,x}$. In this range the log RBE vs log D_n curve should have a slope of $-\frac{1}{2}$.

Although the data in Figs. 1–3 indicate a slope of $-\frac{1}{2}$, it appears that at very high doses the RBE of 0.43 MeV neutrons is likely to be larger than 1. There are also signs of a disagreement at very low doses. For a wide range of site diameters, $\bar{z}_{D,n}/\bar{z}_{D,x}$ for 0.43 MeV neutrons is about 55 (*12*). A dose-independent RBE near this value has in fact been observed at very low effect levels in somatic mutations of *Tradescantia* (*16*), but the data in Fig. 1 indicate that the RBE for lens opacification exceeds this value at the lowest doses investigated. If it is assumed that the experimental findings differ from theory only by a constant factor, one may obtain the fit illustrated in Fig. 1 by multiplication of the right-hand side of Eq. (2) by 4 and choosing the value of 150 rad for $\bar{z}_{D,n}$. The corresponding quantity $\bar{z}_{D,x}$ for x-rays must be less than 5 rad. Sufficient fit of the data is achieved if $\bar{z}_{D,x}$ is neglected, and in this case Eq. (2) reduces to:

$$\text{RBE} = 4\sqrt{1 + (150/D_n)} \tag{3}$$

where the neutron dose D_n is measured in radians. As can be seen from Fig. 1 there is very little latitude in the choice of the two numerical quantities involved. For the other neutron energies the results are subject to considerable statistical uncertainty and the numerical relations are therefore not given.

There may be a connection between the need for a vertical shift of the theoretical curve and the finding that the murine lens is anoxic (*17*). No other detailed attempt has been made to apply the theory to anoxic systems, and it is possible that the constant k in Eq. (1) is not equal for x-rays and neutrons in this environmental condition or that some other modification is required. It may well be that oxygenation influences the effectiveness of radiation, depending on energy concentrations over distances far smaller than the micrometer scale which seems to be the relevant one in RBE effects. The answers to these questions must await the completion of further research.

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