BIOLOGICAL RESPONSES TO VARIOUS NEUTRON ENERGIESFROM 1 MeV to 600 MeV

2. Lens opacification in mice

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ABSTRACT

Eye lens opacification in mice has been used to evaluate the RBE of neutrons of 1, 5, and 600 MeV. The experimental observations were based on counting the points of opacities appearing in the posterior region of the lens. RBE values have been calculated with reference to the equal effects observed after exposure to 250 kVp X-rays. Data previously reported for 15 and 400 MeV neutrons have also been included. It is found that for all neutron energies the RBE increases with decreasing dose. In addition, at very low doses the RBE appears to reach about the same value independently of the neutron energy used. On the contrary, for higher dose values the RBE was found to decrease with increasing neutron energy from about 24 for 1 MeV to about 6 for the 600 MeV neutrons.

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INTRODUCTION

The mouse eye lens represents a biological system which is especially well suited for investigating the effects of neutron irradiations at low doses. However, because of the very long observation time involved, results of measurements using this biological system are scarce. Bateman et al.^{1,2)} published results of mouse lens opacification produced by neutrons of energies between 0.43 and 14 MeV. More recently, Di Paola et al.^{3,4)} reported the results of irradiations with neutrons of 400 MeV and 15 MeV, and with X-rays. In the present paper the results obtained from irradiations with 1, 5, and 600 MeV neutrons are presented to complete the data.

An analysis of the complete set of data has been made. This elaboration enables the RBE dose relationship to be estimated down to the low dose range, where lens opacification was found to be a very suitable test owing to its high radiosensitivity.

The experimental observations based on the counting of the points of opacities in the posterior region of the lens allow us to obtain a fairly quantitative determination of the damage. In addition, the incidence of opacities appearing in the anterior region of the lens was observed, and RBE values for 50% effect were evaluated and compared to the more accurate RBE results determined from posterior opacity observations.

DOSIMETRY

The 600 MeV neutron beam was produced by the CERN 600 MeV Synchro-cyclotron, by irradiating a Be target with 600 MeV protons. Details of the beam characteristics and of the dosimetry used have been reported elsewhere⁵⁾. The dose to the eyes was estimated, using a thin-walled parallel-plate ionization chamber of 60×3 mm with a mylar window of 3.4 mg/cm^2 . The mice were irradiated in individual cages, head-on in the beam, in groups of 20 at a time. As little material as possible was present in front of the biological target; the over-all depth of the eye lens (including the mouse container) was estimated to be 120 mg/cm^2 .

The 1 MeV and 5 MeV neutrons were produced by the 3 MeV Van de Graaff generator located at the Joint Research Centre (JCR), Euratom, Ispra. Details of the beam characteristics and dosimetry used have been reported in a previous paper⁶⁾. In this case also, the mice were irradiated head-on, positioned in a circle at an angle of 25° with respect to the ion beam direction. Enough attenuating material was present around the head of the mice to ensure secondary particle equilibrium.

MATERIALS AND METHODS

Male mice, F1 hybrid between C3H δ and C57Bl δ , 6-7 weeks old, were used for these experiments. At least two weeks before irradiation all animals were transported to the different irradiation facilities used, in order to assure that they became acclimatized. All animals were then kept under standard housing conditions until the end of their life span. They were divided into groups of five per cage, four of which were irradiated and randomly selected per dosage. The animals were periodically weighed. Observations of the opacities were made using a slit-lamp microscope after dilation of their pupils with 1% homatropine.

The number of opacities observed in the posterior region of the lens was recorded separately for each eye. The observer did not know the identity of the animals, and the reproducibility was checked by serial counting of the same group of animals as reported in a previous paper³⁾. All the points of opacity were scored independently of their location and size. The possibility of merging of the individual points when a large number of opacities was present, limit the observations of damage to a total of about 150 points in both eyes.

RESULTS

The number of spontaneous opacities observed in the posterior region of the lens in all non-irradiated animals over a period of 20 months from the simulated irradiations is shown in Fig. 1. Here, as well as in the following figures, error bars represent the standard error of the mean values. The differences found between the controls led to an analysis of the data after subtracting the background opacification. This made it possible to compare results between experiments carried out over a period of several years.

Data from irradiation with X-rays carried out at different times were found to be reproducible, so that data from these experiments could be combined. The experimental points were fitted to a linear regression line. Figure 2 shows the data obtained for 1 MeV neutron irradiation as a function of time for different doses. For this neutron energy, the points above which the method cannot be safely used are already reached eight months after irradiation for the highest dose. At low doses a linearity with time in the progression of the measured points is observed only from eight months after irradiation. The data obtained after irradiation with 5 MeV neutrons are shown in Fig. 3. The error bars for each observation represent the standard error of the mean value. The results obtained after irradiation with 600 MeV neutrons are shown in Fig. 4.

All neutron data obtained are presented in Table 1, where also the number of animals used per dose, the number of opacities observed at the various times of observation, the standard deviations, and the doses are reported.

Linear regression lines and dose-effect curves, have tentatively been fitted to the experimental points for each time of observation. This is illustrated in Fig. 5 for the observations made 11 months after the irradiation of the animals to 1, 5, and 600 MeV neutrons. No subtraction of the number of opacities observed in the control animals has been made prior to the determination of the regression coefficients.

X-rays of 250 kVp were used as the reference radiation. The RBE value for each neutron energy was calculated as an equal effect ratio by referring each neutron dose at each observation time to the corresponding X-ray dose-effect linear regression line, after subtraction of the values for non-irradiated animals. The RBE values obtained at the various times of observation were then averaged for each neutron dose. A similar analysis of the data obtained using 15 and 400 MeV neutrons was also made. The experimental data for these two energies have been published earlier^{3,4}). RBE values are presented in Table 2 for all neutron energies. Those reported in columns 1 and 2 have been obtained by using only the points corresponding to a number of opacities less than 120, whereas the RBE values

reported in columns 3 and 4 are calculated using all the observed values of opacities up to 150, as explained before. Columns 2 and 4 give the values weighted by considering the standard errors of the single points. As can be seen from the table, a significant difference in the RBE values is obtained at the higher doses if opacities up to 150 are used instead of 120. This fact indicates that for the higher doses the possibility of overlapping is non-negligible.

An analysis of the incidence of the opacification in the anterior part of the mice lens 20 months after irradiation was made upon subtraction of the incidence of opacification found in the control animals. Comparisons were made with the X-ray irradiated animals and the RBE values determined. These are reported in Table 3. No information is available from the irradiation with 600 MeV neutrons because of premature death of the irradiated and non-irradiated animals. The RBE values obtained from the anterior opacification appear to be in agreement with those estimated for the posterior region of the eye lens.

The RBE values reported in Table 2 are graphically shown in Fig. 6. It can be seen that the 1 MeV neutrons are the most efficient of the neutron energies studied, followed by the 5, 15, 400, and 600 MeV neutrons. The attenuated 400 MeV neutron radiation, after 18 g/cm² of polyethylene, shows the lowest RBE values; this may be explained by the large contribution to the dose from low LET particles, protons, and γ -rays. For all neutron energies the RBE is shown to depend on the dose, namely decreasing with increasing dose values.

DISCUSSION

The study of lens opacification in mice by using the method of counting the points of opacities present in the posterior region of the lens, has been found to be quantitatively valid and useful for analysing the effect of small doses of radiation. The linear increase of this opacity with time, which persists long after irradiation, suggests that this type of lesion may not be caused by the death of the epithelial cells of the lens, but is more probably a radiation-induced non-lethal damage to these cells resulting in a defective fibre differentiation. A similar hypothesis was already made by Pirie⁷⁾ to explain radiation effects on chicken and rabbit lens.

The RBE variations with dose seem to confirm the over-all trend of data reported in the literature by Bateman et al.²⁾. However, the RBE increase at lower doses is less pronounced for the lowest energy considered. No data exist in the literature for very high energy neutron beams, and from the present experiment no results are available for the 400 MeV neutrons at doses below 0.05 Gy. The results for 600 MeV neutrons show the largest increase of RBE with decreasing doses among the neutron energies studied.

The result that the RBE values evaluated in this study seem to approach similar values when the dose is as low as 0.01 Gy (Fig. 6), regardless of the energy of the neutrons used, may be important for radiation protection. A constant RBE at low doses may be interpreted as being due to a limited number of highly sensitive epithelial cells all of which show the non-lethal effect of opacity formation. Their radiosensitivity is such that a sufficiently high ionization density in the region of a sensitive site is enough to induce the damage regardless of the quality of the neutron beam. By increasing the neutron dose, an increasing number of cells, with lower radiosensitivity, will be injured by the radiation. Thus, differences in the local density of ionizations produced by equal doses of neutrons of different energies may become effective for the formation of the biological damage. As a consequence, RBE values at higher doses will also show a dependence on neutron energy.

In any case, our observation that the RBE approach similar values at a dose of 0.01 Gy proves once again the urgent need to extend the investigation of biological effects to the very low doses that are of interest in radiation protection, using a large range of neutron energies.

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Table 1

Dose (Gy)	3½ months after irradiation			5 months after irradiation			6 months after irradiation			8 months after irradiation			11 months after irradiation			13 months after irradiation			15 months after irradiation			18 months after irradiation			20 months after irradiation		
	No. of mice	No. of opacities (Mean ± s.d.)		No. of mice	No. of opacities (Mean ± s.d.)		No. of mice	No. of opacities (Mean ± s.d.)		No. of mice	No. of opacities (Mean ± s.d.)		No. of mice	No. of opacities (Mean ± s.d.)		No. of mice	No. of opacities (Mean ± s.d.)		No. of mice	No. of opacities (Mean ± s.d.)		No. of mice	No. of opacities (Mean ± s.d.)		No. of mice	No. of opacities (Mean ± s.d.)	
600 MeV neutrons																											
Controls	45	9.8	3.2	45	10.7	3.0	45	11.2	2.7	43	14.9	3.3	42	30.5	5.6	42	40.5	6.6	42	58.3	9.9	42	72.2	8.6	34	86.9	14.9
0.016	20	10.0	2.6	20	12.7	4.9	20	14.7	4.1	20	19.1	3.8	20	35.3	7.4	20	46.3	9.9	20	58.9	13.6	19	73.8	10.8	16	87.2	12.5
0.046	19	11.0	3.3	19	12.5	3.3	19	13.6	3.5	18	20.2	6.5	18	43.6	10.0	18	58.8	14.0	17	72.0	20.8	14	93.1	14.0	12	110.4	15.4
0.086	19	12.3	3.9	19	15.5	4.5	19	17.6	4.2	18	23.9	3.2	18	46.4	7.9	18	56.9	12.1	18	71.8	11.9	17	89.5	15.2	14	98.4	16.6
0.166	17	12.7	2.9	17	18.1	4.1	17	25.7	6.2	17	32.2	4.9	17	56.1	10.2	17	73.3	12.5	17	90.8	11.0	15	105.0	10.9	11	125.2	12.0
0.296	19	15.7	4.0	19	24.7	5.2	19	32.5	6.6	19	43.8	7.9	19	72.4	11.1	19	88.3	15.2	19	108.5	21.8	17	130.5	13.9	15	146.1	16.4
0.406	19	17.6	5.2	19	28.9	5.3	19	38.8	6.8	19	52.5	6.5	19	86.1	12.1	18	108.4	15.4	16	131.4	16.2						
	4 months			6 months			8 months			11 months			13 months			18 months			20 months								
5 MeV neutrons																											
Controls	44	8.7	2.9	44	10.8	2.9	44	14.6	3.8	44	26.4	5.7	44	39.7	6.5	42	67.5	9.0	39	73.2	9.1						
0.009	17	8.3	3.1	44	12.0	3.5	17	15.8	4.7	17	29.1	10.2	16	44.7	10.2	15	72.1	8.8	15	78.9	9.2						
0.046	19	12.5	3.0	19	17.6	4.7	19	23.8	6.4	19	40.1	7.7	19	59.7	13.6	18	91.9	13.5	16	98.3	10.7						
0.076	12	13.1	4.3	12	22.6	4.8	12	37.4	8.1	12	50.2	12.0	12	72.9	17.1	12	112.1	16.1	12	118.8	17.1						
0.116	8	15.4	3.8	8	30.5	8.1	8	41.4	3.7	8	57.5	8.3	8	80.7	19.3	8	120.2	15.7	8	128.4	16.6						
0.156	12	18.2	4.0	12	35.5	8.6	12	46.7	10.3	12	76.2	14.5	12	102.0	21.5	10	122.0	22.1	10	140.6	20.4						
0.236	19	24.9	8.2	19	48.3	13.7	19	64.3	13.9	19	97.0	15.8	19	123.2	20.6												
0.356	8	37.4	5.5	8	69.0	9.4	8	84.5	9.9	8	118.1	15.9	8	142.5	9.9												
1 MeV neutrons																											
Controls	44	8.7	2.9	44	10.8	2.9	44	14.6	3.8	44	26.4	5.7	44	39.7	6.5	42	67.5	9.0	39	73.2	9.1						
0.009	20	9.7	3.5	20	13.5	4.3	20	19.3	6.3	20	33.0	6.2	20	48.2	9.1	17	75.3	13.0	17	85.1	14.8						
0.056	20	16.4	4.1	20	25.8	7.1	20	35.6	8.4	20	49.9	8.4	20	74.8	10.5	19	105.8	18.5	19	116.5	19.9						
0.116	20	20.1	6.0	20	39.5	9.6	20	57.1	10.7	19	87.6	10.4	19	110.0	16.8	18	143.6	14.0									
0.22	20	40.6	7.3	20	86.7	9.9	20	107.9	17.0	20	143.9	16.6															
0.356	20	49.2	7.0	20	100.2	16.1	20	126.0	20.1																		

Table 2

Mouse lens opacification
Average RBE values

Dose (Gy)	RBE ₁ unweighted	Standard errors of the mean	RBE ₂ weighted	Standard errors of the mean	RBE ₃ unweighted	Standard errors of the mean	RBE ₄ weighted	Standard errors of the mean
1 MeV neutrons								
0.009	40.7	±3.8	41.9	±3.4	40.7	±3.8	41.9	±3.4
0.056	23.5	±0.8	23.8	±0.8	23.5	±0.8	23.8	±0.8
0.116	24.5	±0.5	24.4	±0.4	24.1	±0.5	24.0	±0.5
0.220	24.5	±1.0	24.5	±1.0	23.7	±0.8	23.8	±0.8
0.356					18.1	±0.8	18.1	±0.80
5 MeV neutrons								
0.009	24.5	±3.1	24.0	±2.9	24.5	±3.1	24.0	±2.9
0.046	17.3	±0.7	17.1	±0.7	17.3	±0.7	17.1	±0.7
0.076	18.4	±0.8	18.6	±0.8	18.4	±0.8	18.6	±0.8
0.116	14.9	±0.5	15.1	±0.5	14.9	±0.4	15.1	±0.4
0.156	14.5	±0.7	14.3	±0.7	13.6	±0.7	13.6	±0.6
0.236	13.1	±0.3	13.1	±0.3	13.1	±0.2	13.1	±0.2
0.356	11.5	±0.2	11.5	±0.1	11.1	±0.3	10.9	±0.4
15 MeV neutrons								
0.010	33.6	±2.4	33.4	±2.3	33.6	±2.4	33.4	±2.3
0.040	14.5	±0.5	14.2	±0.5	14.5	±0.5	14.2	±0.5
0.076	11.5	±0.5	11.5	±0.4	11.5	±0.5	11.5	±0.4
0.165	9.3	±0.8	9.3	±0.8	9.4	±0.3	9.4	±0.3
0.310	9.0	±0.5	9.0	±0.4	8.9	±0.3	8.9	±0.3
0.380	9.0	±0.7	9.5	±0.6	9.0	±0.4	9.3	±0.5
400 MeV neutrons								
0.050	7.3	±0.3	7.2	±0.3	7.3	±0.3	7.2	±0.3
0.090	8.5	±0.4	8.6	±0.3	8.5	±0.4	8.6	±0.3
0.190	6.1	±0.1	6.1	±0.1	6.1	±0.1	6.1	±0.1
0.380	6.0	±0.2	5.9	±0.1	5.9	±0.4	5.8	±0.4
400 MeV neutrons attenuated beam								
0.070	5.0	±0.4	4.9	±0.5	5.0	±0.4	4.9	±0.5
0.150	2.2	±0.4	2.3	±0.3	2.2	±0.4	2.3	±0.3
0.270	2.8	±0.1	2.8	±0.1	2.8	±0.1	2.8	±0.1
0.580	2.3	±0.1	2.3	±0.1	2.3	±0.1	2.3	±0.1
600 MeV neutrons								
0.016	21.6	±7.2	25.7	±7.0	21.6	±7.2	25.7	±7.0
0.046	15.8	±1.4	15.0	±1.6	15.8	±1.4	15.0	±1.6
0.086	7.4	±0.9	7.8	±0.7	7.4	±0.9	7.8	±0.7
0.166	7.1	±0.3	7.1	±0.3	7.1	±0.2	7.0	±0.2
0.296	6.3	±0.2	6.2	±0.2	6.2	±0.1	6.2	±0.1
0.406	5.9	±0.2	5.8	±0.2	5.9	±0.2	5.8	±0.2

Table 3

RBE for 50% incidence of anterior opacities
after subtraction of controls

Neutron energy (MeV)	RBE
1	19
5	9
15	6
400	6

Figure captions

- Fig. 1 : Spontaneous opacities in unirradiated mice.
- Fig. 2 : Number of opacities observed after irradiation to various doses of 1 MeV neutrons.
- Fig. 3 : Number of opacities observed after irradiation to various doses of 5 MeV neutrons.
- Fig. 4 : Number of opacities observed after irradiation to various doses of 600 MeV neutrons.
- Fig. 5 : Number of opacities observed, as function of dose, 11 months after exposure to 1 MeV, 5 MeV, and 600 MeV neutrons. (Background opacities observed in controls have not been subtracted).
- Fig. 6 : Variation of RBE with dose for the various neutron energies used.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6