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HS Division Report

LENS OPACIFICATION IN MICE EXPOSED TO 14 MeV NEUTRONS

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1. INTRODUCTION

Radiation induced lens opacification in mice has been the subject of extensive studies, in particular with fast neutrons¹⁻³). The reason for this has been to verify the early observation of cataract in cyclotron workers who had the habit of holding fluorescent material in the path of an 8-16 MeV deuteron or proton beam in order to locate its position. It was assumed, at the time, that the human lens was particularly sensitive to high LET radiation, but a more recent review of this problem has failed to prove this assumption, and consequently the maximum permissible exposure to the eye lens has been changed accordingly⁴).

In spite of the fact that the biological characteristics of the eye lens of mice are different from those of the human eye, it offers interesting aspects for a study of radiobiological effects. First of all, the lens is a biologically well-defined organ, located at the surface, and is, in addition, of a sufficiently small size such that homogeneous exposures may be achieved even if exposed under conditions of a substantial build-up of dose with depth. The epithelium of the eye lens in mice is in a proliferative and differentiative state at a declining rate during the whole lifetime of the animal. It consists of cell proliferative tissue, which differentiates into fibres surrounded by a hyaline capsule that prevents penetration of cells from the outside and consequently a dispersion of radiation effect.

Lens opacification in mice has already been studied for a neutron beam with a maximum energy of 400 MeV⁵). Therefore, some interest exists into studying this particular effect from 14 MeV neutrons where the nuclear processes, creating heavily charged secondary particle radiation, are absent. The absorbed dose of high LET in the case of 14 MeV neutrons is mainly due to recoil protons. In this paper the results from a study of 14 MeV neutron radiation are summarized and compared with the effects of 250 kVp X-rays. This has been done in order to establish the RBE values from the observation of lens opacification in mice.

2. THE EXPERIMENTAL SET-UP

The experimental set-up used for the exposure of the animals is shown in Fig. 1. 14 MeV neutrons were produced by the SAMES neutron generator^{*)} and the mice were irradiated, head on, inside polythene test tubes with thin windows (120 mg/cm²). The eyes were located 60 cm from the target of the neutron generator. 14 MeV neutrons were produced by the (d,t) reaction, using a deuterium beam of 150 keV. The average energy of the neutrons was estimated to be 14.75 MeV, due to some attenuation in the target itself.

*) CEA, Grenoble.

3. DOSIMETRY

The dose absorbed by the eye lens of the mice was determined by three different ionization chambers of parallel-plate design of different sizes. They were all made of tissue-equivalent material and filled with tissue-equivalent gas.

One chamber had a diameter of 2 cm with an electrode spacing of 2 cm, another had a diameter of 6 cm and a spacing of 0.3 cm, and the third one was of the same size but with a mylar window of 2 mg/cm². All three chambers were utilized to determine the absorbed dose at a depth of 100 mg/cm², which was considered to be the depth of the eye lens in the mice.

The dose was determined from the observation of the ionization current I and by applying a calibration figure k , obtained from measurements with a known ⁶⁰Co source. The correction factors used are given in the equation:

$$D = \frac{I}{P} k f_w f_c f_d f_{comp} ,$$

where P is the gas pressure, f_w the correction factor for w , f_c the response difference between γ and neutron radiation, f_d the depth correction factor, and f_{comp} the correction factor to convert from tissue-equivalent material to the composition of the eye lens. The dose estimate was made by using the following values for these various factors: $f_w = 1.03$, $f_c = 0.99$, f_{comp} by assuming the eye lens consisting of 80% H₂O and 20% soft tissue. The dose was determined at a depth of 100 mg/cm² using the measured depth-dose distribution shown in Fig. 2. From the three independent determinations of the dose absorbed by the eye of the mice, values were obtained which agreed within $\pm 4\%$. In addition, and as an independent control, the dose was calculated from fluence measurements made with activation of discs of S and Al. Both these detectors gave results which agreed within 3%. A flux-to-absorbed dose conversion factor of 6.22×10^{-9} rad/n/cm² for the eye lens was used. This value was in close agreement with calculations⁶⁾.

During all exposures a fission neutron counter was used as beam monitor. This was located at a fixed position with respect to the target of the neutron generator. The doses given were 0.95, 4, 7.6, 16.5, 31, and 38 rad. A split-dose irradiation of 7.1 + 7.5 rad was given 24 h apart. The dose-rate was 6.5 rad/h for all exposures.

X-ray irradiations were done at Casaccia (Italy) using a 250 kVp X-ray set with 15 mA filters of 2 mm Pb, 1 mm Cu, 1 mm Al (HVL 5 mm Cu). A dose-rate of 45.8 rad/h, at a distance of 80 cm from the target, was applied. The animals were exposed to doses of 20, 40, 60, 80, 120, and 160 rad. Split-dose irradiations of 40 + 40, 60 + 60, and 80 + 80 rad, given 24 h apart, were also performed.

4. MATERIALS AND METHODS

Male mice hybrid between C57Bl ♀ and C3H ♂, with an average age of 75 days at the time of exposure, were used for this experiment. The animals were flown from Rome to Geneva and transported two weeks later to Grenoble, where the irradiation by the 14 MeV neutrons took place. After this irradiation the animals were transported back to Rome via the same route.

The mice were kept in groups of five per cage and maintained under standard conditions at the Cassacia animal house for the rest of their life. In each cage one unirradiated mouse was mixed with four irradiated mice chosen at random.

The eye lenses of the animals were examined periodically with a slit-lamp microscope after dilation of the pupils with 1% homatropine. The body weight was checked at the same time. All the observations were made by the same examiner who had no prior knowledge about the identity of the animals.

The scoring of the number of opacities seen on the posterior part of the lens was made disregarding the size of each spot, and by reporting the sum of the number of spots observed in the left and right eye. A good reproducibility was achieved by the examiner for the low degree of opacity. When the opacities increased in number and began to coalesce, this method could no longer be used. The incidence of the opacities in the anterior part of the lens was then evaluated.

5. RESULTS

The number of observed opacities in the posterior region of the lens at 3, 4, 6, 8, 11, 13, 15, 18, and 20 months after irradiation are reported in Table 1. The numbers indicate the mean of the observed opacities with the standard deviation.

Observation of the various unirradiated groups of animals showed no difference in the progression of the lens opacification with age. This confirms the good reproducibility of this particular biological test, also reported by other authors⁷⁾. In Fig. 3 are shown the results of the observed spontaneously appearing opacities in 247 mice as a function of their age. This is characterized by an initial slow linear progression up to the age of 10-11 months, after which the progression is almost 3 times as fast.

Due to the very good reproducibility it was decided to pool two independent experiments with 250 kVp X-rays done at different times. These results are shown in Fig. 4. Figure 5 shows the results obtained after exposure to 14 MeV neutrons. These two graphs indicate a general linear progression of the observed opacities with age as for the control groups, both indicating an increasing slope with dose. The lines drawn have been calculated from the points using the least squares fit method.

The number of opacities as a function of dose gives a linear dose effect relation as shown in Figs. 6 and 7, where the results from the observations at 8 months and 18 months are reported.

Calculated regression lines from the experimental points give RBE values between 8 and 9 when the 250 kVp X-ray results are used as reference values. Determination of RBE values by comparing doses that produce the same number of opacities gives values ranging from 9 to 21, depending on the dose given. This is illustrated in Figs. 8a and 8b.

The per cent incidence of lens opacities of the anterior region of the eyes at 20 and 25 months after irradiation is shown in Fig. 9. An RBE value of eight gives the best superposition of the X-ray and 14 MeV neutron results in this case.

A split-dose investigation was carried out by applying the dose in two fractions, 24 h apart. The results are presented in Table 1. Compared to the single-dose exposures a difference appears in the observed effect of the split X-ray dose, whereas in the case of 14 MeV neutrons no such difference is observed.

6. DISCUSSION

The progression of opacities with time, which is found to be linear and dose-dependent, suggests that the induction of opacification in mice is not a cell-killing process but rather a result of some disturbances of the differentiation of cells into fibres.

The method of counting the individual number of opacities, appearing on the posterior part of the eye lens, is a sufficiently sensitive method to evaluate the lens opacification in mice at low doses. This is similar to the fleck-counting method applied by Bateman and Snead⁸⁾, which has yielded RBE values reaching six for a dose of 4.8 rad of 14 MeV neutrons. The RBEs observed in this work agree well with their values and confirm, in addition, the strong RBE dependence on dose for 14 MeV neutrons, but yields a lower value than the one reported by Bateman and Snead for low comparable dose values.

If the RBE values of 14 MeV are compared with those we have reported previously for 400 MeV neutrons, it is concluded that 14 MeV neutrons are generally more effective in producing lens opacification. The RBE observed for 400 MeV neutrons was 5.8 for the posterior region of the lens and 5.4 for the anterior part. In the case of 400 MeV neutron radiation no RBE dose dependence between 5 and 38 rad was observed.

Other similar experiments have, however, to be carried out, both at higher and lower neutron energies, in order to confirm and extend the present knowledge of the RBE dependence on neutron energy and dose. The difference observed between the

effects of a split dose, 24 h apart, is in general agreement with the values reported in the literature for lower energy neutrons and might indicate the lack of repair in the case of high LET radiation⁹⁾.

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Table 1
Number of opacities in the posterior region of the lens

Dose (rad)	3 months after irradiation		4 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	Mean $\pm$ SD	No. of mice	Mean $\pm$ SD	No. of mice	Mean $\pm$ SD	No. of mice	Mean $\pm$ SD	No. of mice	Mean $\pm$ SD	No. of mice	Mean $\pm$ SD	No. of mice	Mean $\pm$ SD	No. of mice	Mean $\pm$ SD	No. of mice	Mean $\pm$ SD
250 kVp X-rays																		
Controls	26	6.0 $\pm$ 1.4	26	7.1 $\pm$ 1.8	26	8.6 $\pm$ 1.9	26	14.0 $\pm$ 3.1	26	25.9 $\pm$ 4.3	25	35.6 $\pm$ 4.1	25	45.4 $\pm$ 5.9	25	57.5 $\pm$ 6.1	25	67.7 $\pm$ 7.1
20	20	6.6 $\pm$ 1.3	20	7.5 $\pm$ 1.3	20	9.1 $\pm$ 1.8	20	16.0 $\pm$ 3.7	20	29.6 $\pm$ 5.2	20	40.2 $\pm$ 6.8	19	48.3 $\pm$ 8.2	16	61.8 $\pm$ 9.9	15	73.6 $\pm$ 6.0
40	20	7.1 $\pm$ 1.7	20	8.4 $\pm$ 1.7	20	10.2 $\pm$ 2.3	20	17.6 $\pm$ 3.8	20	35.4 $\pm$ 7.1	20	44.6 $\pm$ 6.2	20	55.6 $\pm$ 8.3	20	67.8 $\pm$ 9.2	19	77.6 $\pm$ 9.4
60	20	7.2 $\pm$ 1.5	19	8.8 $\pm$ 1.6	19	12.3 $\pm$ 3.0	19	21.8 $\pm$ 5.1	19	37.1 $\pm$ 7.7	19	48.9 $\pm$ 6.5	19	64.3 $\pm$ 9.7	17	77.4 $\pm$ 11.4	16	87.4 $\pm$ 12.9
80	20	8.1 $\pm$ 1.8	20	9.8 $\pm$ 1.9	20	16.6 $\pm$ 3.9	19	25.4 $\pm$ 5.2	19	42.4 $\pm$ 7.4	18	55.3 $\pm$ 8.4	18	77.6 $\pm$ 10.4	17	79.3 $\pm$ 8.4	16	91.7 $\pm$ 10.4
120	20	8.9 $\pm$ 1.7	20	11.6 $\pm$ 2.4	20	19.6 $\pm$ 4.3	19	32.7 $\pm$ 7.8	19	52.3 $\pm$ 6.6	19	65.3 $\pm$ 8.7	18	79.9 $\pm$ 10.6	18	98.5 $\pm$ 13.1	16	112.3 $\pm$ 14.9
160	20	11.2 $\pm$ 1.3	20	14.0 $\pm$ 2.8	20	26.8 $\pm$ 5.3	19	41.4 $\pm$ 7.0	19	62.8 $\pm$ 7.6	19	74.6 $\pm$ 5.8	18	87.4 $\pm$ 7.6	18	108.6 $\pm$ 9.8	18	123.9 $\pm$ 11.0
14 MeV neutron beam																		
Controls	36	6.6 $\pm$ 1.7	36	7.5 $\pm$ 1.8	34	8.5 $\pm$ 2.2	34	13.5 $\pm$ 3.2	34	24.6 $\pm$ 5.1	34	36.3 $\pm$ 5.6	34	47.1 $\pm$ 6.0	31	57.7 $\pm$ 5.9	28	72.2 $\pm$ 5.2
0.95	20	7.0 $\pm$ 1.5	20	7.9 $\pm$ 1.2	20	9.1 $\pm$ 2.0	20	16.6 $\pm$ 5.7	20	28.2 $\pm$ 4.3	20	41.0 $\pm$ 6.3	20	52.9 $\pm$ 6.5	19	69.9 $\pm$ 11.5	18	84.2 $\pm$ 9.5
4.0	20	7.7 $\pm$ 1.6	20	9.0 $\pm$ 2.4	19	12.9 $\pm$ 2.9	19	20.7 $\pm$ 4.7	19	36.5 $\pm$ 8.0	19	48.2 $\pm$ 8.4	19	58.5 $\pm$ 8.1	19	77.7 $\pm$ 12.0	19	93.3 $\pm$ 12.6
7.6	20	8.6 $\pm$ 1.9	20	10.5 $\pm$ 3.1	20	17.1 $\pm$ 4.9	19	28.6 $\pm$ 6.5	19	43.9 $\pm$ 6.9	19	56.7 $\pm$ 7.1	19	68.1 $\pm$ 8.5	19	82.9 $\pm$ 12.6	17	102.5 $\pm$ 8.7
16.5	19	10.4 $\pm$ 2.3	19	13.8 $\pm$ 2.4	18	26.8 $\pm$ 5.3	18	39.8 $\pm$ 8.0	18	55.8 $\pm$ 10.8	18	73.9 $\pm$ 10.1	18	89.5 $\pm$ 12.2	16	111.1 $\pm$ 11.9	16	129.1 $\pm$ 11.3
31	20	12.6 $\pm$ 2.4	20	18.9 $\pm$ 4.3	20	41.0 $\pm$ 5.4	20	65.9 $\pm$ 12.4	19	84.9 $\pm$ 12.2	19	109.9 $\pm$ 12.0	19	125.7 $\pm$ 13.0	19	142.7 $\pm$ 10.7	17	-
38	20	14.0 $\pm$ 2.2	20	23.4 $\pm$ 5.1	19	49.6 $\pm$ 4.3	19	78.2 $\pm$ 8.9	19	99.4 $\pm$ 14.2	19	128.3 $\pm$ 14.0	17	143.8 $\pm$ 11.0	16	-	15	-
Split dose (half dose 24 h apart)																		
X-rays																		
40+40	19	7.4 $\pm$ 1.8	19	9.9 $\pm$ 2.6	19	15.0 $\pm$ 3.2	19	23.4 $\pm$ 4.9	19	37.6 $\pm$ 6.2	19	48.6 $\pm$ 6.4	19	60.8 $\pm$ 10.0	18	75.4 $\pm$ 7.2	18	85.3 $\pm$ 10.7
60+60	20	7.9 $\pm$ 1.8	19	10.9 $\pm$ 2.7	19	17.8 $\pm$ 5.6	19	28.2 $\pm$ 5.0	19	46.6 $\pm$ 6.7	19	59.0 $\pm$ 7.0	18	74.2 $\pm$ 10.2	18	85.0 $\pm$ 8.9	14	99.4 $\pm$ 14.5
80+80	18	8.8 $\pm$ 2.3	18	11.1 $\pm$ 2.2	18	20.6 $\pm$ 6.4	18	34.7 $\pm$ 7.0	17	55.2 $\pm$ 8.0	17	67.9 $\pm$ 7.6	17	80.5 $\pm$ 9.8	17	101.0 $\pm$ 9.2	16	110.2 $\pm$ 9.9
neutrons																		
7.1+7.5	20	9.4 $\pm$ 1.4	20	12.4 $\pm$ 2.8	19	23.2 $\pm$ 5.1	19	37.9 $\pm$ 6.9	18	55.7 $\pm$ 9.7	18	74.1 $\pm$ 9.9	17	84.2 $\pm$ 11.3	15	101.7 $\pm$ 8.7	14	118.7 $\pm$ 10.8

Figures captions

- Fig. 1 : Irradiation set-up.
- Fig. 2 : Depth-dose distribution of 14 MeV neutrons.
- Fig. 3 : Spontaneous lens opacities appearing with age in unirradiated mice.
- Fig. 4 : Number of lens opacities in mice observed after irradiation with X-rays.
- Fig. 5 : Number of lens opacities in mice observed after irradiation with 14 MeV neutrons.
- Fig. 6 : Number of lens opacities in mice as a function of dose after exposure to 250 kVp X-rays and 14 MeV neutrons 8 months after irradiation.
- Fig. 7 : Number of lens opacities in mice as a function of dose after exposure to X-rays and 14 MeV neutrons 18 months after irradiation.
- Fig. 8 : Doses of neutrons and X-rays producing the same number of lens opacities in mice as a function of time after exposure.
- Fig. 9 : Incidence of opacities in the anterior region of the lens of mice 20 months and 25 months after irradiation as a function of dose (neutrons and X-rays).

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6

Fig. 7

Fig. 8

Fig. 9