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LENS OPACIFICATION IN MICE EXPOSED TO
MONOENERGETIC FAST NEUTRONS*

by

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MASTER

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INTRODUCTION

Early effects obtained with monoenergetic fast neutrons in mice have been described elsewhere (1,2,3). Emphasis in this report will be placed on the late effect of lens opacification (cataractogenesis), particularly during the period soon after irradiation with low or fractionated doses of neutrons at two energy levels, or X-rays. Consideration will also be given to the influence of age at time of irradiation upon the induction of lens opacities. Both studies are continuing, with periodic slit-lamp microscope examinations, but findings to date warrant this initial report at this Conference.

Lens opacities resulting from human exposures to ionizing radiation have been of major interest for several decades, particularly with regard to physicists engaged in work where high local doses to the lens with radiations of high linear energy transfer (LET) may occur. Concern has also attended the therapeutic use of ionizing radiation to the eye or head, and more recently, exposures to the fission neutrons resulting from reactor or criticality excursions or nuclear detonations. Experimental interest in radiation cataractogenesis has focused partly on the unique (avascular) physiology of the lens, and partly on the high relative biological effectiveness (RBE) of radiations such as fast neutrons in causing lens opacities, when compared to X- or gamma- rays. RBE values of 4-10, often more than double those for 30 day 50 percent lethality, have been obtained(4,5). Studies of radiation effectiveness in producing opacities have generally involved serial visual examinations with a slit-lamp microscope or ophthalmoscope. Parallel histological evaluations have generally indicated the type of damage to be independent of the LET. The extensive cytologic and

biochemical considerations of radiation changes within the lens will not be treated in this paper, but a recent comprehensive bibliography by Lerman (6) contains numerous references to such work.

Radiation cataractogenesis varies markedly with species, man being among the most resistant of mammals, and mice being usually the most sensitive. Rats, guinea pigs, and rabbits lie between these two extremes, but most studies of radiation effectiveness in cataract production have involved mice. Visual examination for radiation-induced opacities must of necessity involve the use of an arbitrary scale, set up and adhered to by a single observer for any one experiment, with identity of the animals concealed. Figure 1 illustrates the grading employed by Riley et al. (4) on the basis of observations made through an ophthalmoscope. Opacification numbers from 0 through III were assigned according to the degree of opacification, and averages computed for each group of animals. A similar scoring scale was utilized by Upton et al. (5) with one more division, for observations made with a slit-lamp microscope. In each case, and in similar studies, scoring has been primarily concerned, at least in the earlier stages, with changes within the posterior lens substance, although anterior changes have been frequently commented upon, particularly as cataracts approach maturity.

In the present investigations, the degree of lens opacification was studied as a function of time following exposure of mice to low and fractionated doses of monoenergetic fast neutrons. A slit-lamp microscope was employed to allow finer visualization of the lens, and in initial studies it soon became evident that considerable information would be lost if grading of the degree of opacification were limited to 3 or 4 categories, and if a method were not devised to immobilize the head of the mouse

during examination. Thus a finer scoring scale was employed, and a mechanical restrainer was designed and constructed to allow accurate positioning and holding of the mouse during examination. These procedures also allowed a finer differentiation between anterior and posterior lens opacities, which have been often noted to differ markedly in character, onset, and rate of development. It was decided to study also the influence of age at irradiation on the degree of radiation-induced lenticular opacification, because several workers (6,7) have implied an increasing radiosensitivity with decreasing age at irradiation, but no comprehensive studies of this relation in cataractogenesis were found in the literature.

MATERIALS AND METHODS

X-ray exposures were accomplished by means of a 250 kvp Maxitron with a radial-field tube operating at 30 ma, with a HVL=2.5 mm of Cu, an average dose rate of 16.9 rads/minute and a T.S.D.=106 cm. During exposure the mice were enclosed within a 1/4" wall Tygon tube of 1" I.D.; shielding with 1/4" of lead was employed for the partial body exposures. For Co⁶⁰ gamma irradiations, a 1/4" wall lucite box was used, suspended 10" above a moveable carrier with five stainless steel clad cobalt plates totaling approximately 40 curies and yielding an average of 18.3 rads per minute. Monocnergetic fast neutrons were obtained by use of a 3 Mev Van de Graaff generator to accelerate 2.8 Mev protons into a water-cooled tritium target, as described in earlier work (1). Fast neutrons are emitted in all directions from the target, with flux and energy being strong functions of neutron path angle to the beam-target axis. Neutrons of 0.43 Mev were received by mice positioned so their eyes were located on a circle centered on and at right angles to the beam target axis, with a forward angle to the

beam axis of 130° and a target center to mouse eye distance of 10 cm. The mice were fixed individually in 50 ml Lusteroid centrifuge tubes with the long axis located radially and the noses of the mice pointed at the target. Neutrons of 1.80 Mev were received by other groups of mice similarly constrained, but at a forward angle of 20° , and with a target center to eye midpoint distance of 19.9 centimeters. Eight mice, equally spaced, were irradiated in each exposure circle at one time, and replicate exposures within each experiment were necessary. Doses were assumed to be equal to the kerma in tissue at the position of the animals ("first collision dose") using factors derived in previous work. Positions in both exposure circles were provided by pockets in a Styrofoam (expanded plastic) carousel, which rotated on the beam axis at about 0.3 rpm to allow for slight field inequality, and to equalize stress in the mice. Extensive dosimetry is being carried on and doses stated in this report will be subject to modification.

All animals employed were mice of the BNL-Stoner (white Swiss) strain, and were maintained on water and Purina Chow pellets ad libitum, in an environment with controlled light, temperature, and humidity.

The animals used in the study of cataractogenesis as a function of age were treated as shown in Table I. It was necessary to breed mice especially for the 0, 1, and 2 week (age at irradiation) groups, and both males and females were employed. (No subsequent difference was found in degree of opacification between males and females). The five and

eight week groups consisted only of males from the regular colony, and all groups were made available for this series of examinations by other investigators who carried out the exposures for long-range experiments. All mice in the zero and one week groups exposed to 800 rads died prior to the first lens examinations, and a few scattered subsequent deaths have occurred since among the survivors in other groups.

The animals used for the study with low and fractionated doses of fast neutrons or X-rays were females of 15-18 weeks of age at time of treatment. The experimental plan is shown in Table II. All animals survived to the first examination and a few scattered deaths have occurred since.

Periodic examinations for lens opacities have been carried out on the animals in each experiment, employing a technique differing from those described in the literature. The irises are dilated with 1% homatropine and animal identification is not known to the observer until all groups have been examined. The mice are not anesthetized, but each is placed in a double-swiveled holder which allows the A-P axis of each lens to be aligned with the optical axis of the microscope. Figure 2 shows the general setup employed for lens examination. The holder incorporates a forward-tilting feature and an azimuth pivot which permits a rapid switch between examinations of the right and left eyes. A counterweight on the removable mouse platen allows maintenance of any angle with minimum friction. Mice are loaded with the platen lifted off the azimuth pivot, and are restrained by an elastic-loaded bar which locks their shoulders between four vertical pins. Skin of the head and neck can be drawn back slightly beneath this bar, causing the eyes to project outward, which facilitates

their examination. The procedure is usually accepted without struggle by the mice, and the accurate and steady positioning allows rapid and complete study of each lens. This holder is used in conjunction with a Bausch and Lomb slit-lamp microscope, operating at a magnification of 15.5 x, in a room darkened except for the technician's recording desk.

Figure 3 shows the scoring system employed to assign separate values to the regions "A", "V", and "P", for each lens examined. The last designation indicates vitreous opacities which have proven mainly of interest: 1) as markers in the serial examinations, because mice have not been coded within the groups, and 2) for their tendency when large to interfere with scoring of small opacities in the "V" region. The anterior (A) and posterior (V) opacities are of interest separately because of their differing development, and are easily scored separately in the early stages. "A" opacities, when large, can make accurate scoring of the "V" opacities difficult and, to a lesser extent, the converse is true. Sufficient "A" opacification, that is 50% or more, will preclude satisfactory examination of "V" opacities, and such a lens is omitted in calculation of an average "V" score for a group. As indicated in the figure, an opacity in any area is estimated as to size in terms of percent of the lens cross-sectional area. This score may then be adjusted upward or downward, depending on comparison of average optical density of the opacity with an arbitrary standard. The growth of opacities, especially in the "A" area, appears to progress mostly laterally, and accordingly, such scoring provides a fair estimate, not only of opacity area, but also volume (of damaged tissue). "V" opacities, however manifest an appreciable component of their growth by forward extension, and to this extent, are less accurately measured as regards volume. Assay of the degree of opacification should take into account both the

volume of damaged tissue and average degree of damage. The described system is only partially effective in this direction, as is any purely visual examining technique. Accuracy in estimating projected opacity area decreases with increasing size of the opacity, and is reflected by the increased spacing in the "usual scoring" (Figure 3) as one moves from 0 to 100%.

RESULTS

Not all observations obtained are illustrated in the succeeding figures in the interest of clarity and reasonable brevity, but those curves shown are typical of the entire data and demonstrate the pertinent findings.

"P" opacities of appreciable size (i.e. 5% and over) were found initially in about 5% of the mice. These did not change in incidence or size during the period covered by the serial examinations and have been omitted from further consideration.

Figure 4 shows the development with time of "V" opacities after exposure to 500 rads of gamma radiation versus age at irradiation (first experiment). (Unfortunately these animals were not available for study prior to the 20th week post-irradiation, by which time opacities were well started). A major point of interest is the persistently lower incidence of opacities in the mice irradiated at lesser ages. Figure 5 indicates the status of "V" opacities for three doses at 30 weeks post-irradiation, and shows mainly grading of opacity with dose, and a lesser incidence of opacity for both doses in the 0 and 1 week groups. In Figure 6 is seen the serial development of "A" opacities following 500 rads of gamma radiation, with again a lesser incidence of opacification persisting in the 0 and 1 week groups. However, the 8 week group reveals a similar lessened incidence

initially, but a subsequent acceleration of opacification which makes the "A" incidence essentially the same in the 2, 5, and 8 week groups at present. (This "catching up" was also seen with 600 and 800 rad exposures in the 8 week group). Figure 7 shows the status of "A" opacities at 30 weeks post-irradiation for the various doses employed, which conform to curves basically similar to the corresponding curves for "V" opacities although the "percent opacification" is only about one-third as much.

Figure 8 illustrates the progression of "V" opacities following exposure to 0.43 Mev neutrons (second experiment), with an appreciable incidence evident in the one rad group, and no suggestion of a threshold. Fractionation did not significantly reduce "V" opacification. Figure 9 shows comparable results from exposures to 1.80 Mev neutrons, but suggests a slight reduction in opacification with fractionation at the 30 rad level. Development of "V" opacities following irradiation with 250 kvp X-ray is seen in Figure 10, and shows first, the absence of an abscopal effect with head shielding at 550 rads, and second, an appreciable reduction in incidence of opacities with fractionation. Figure 11 compares the status of "V" opacities at 26 weeks post-treatment for all three types of radiation. It appears that 0.43 Mev neutrons are slightly more effective than those of 1.80 Mev, and that both are about ten times as effective as 250 kvp X-ray, in the dose range covered. In this experiment "A" opacities with neutrons are just becoming significant with exposures to neutrons of either energy, as seen in Figure 12, but are too low at present to allow interpretation. With regard to the X-ray data, this

curve alone shows a marked difference in curvature, from all preceding plots, including that for the incidence of "V" opacification with X-ray. Fractionation of the 975 rad dose appears to have significantly reduced the incidence of "A" opacities, in a degree comparable to that seen for "V" opacities. In this second experiment, it has been of interest to note the fairly regular progression of opacification on dose with the low doses employed. It should be pointed out that the number of animals was doubled in the control group, and in the lowest dose group for each radiation employed, in the interest of clarifying the question of an opacification threshold.

DISCUSSION

Both of the experiments described are continuing, and thus firm conclusions at this time are not warranted. In particular, the possibility of opacities becoming stationary or even regressing must be considered at the lower doses of both neutron and X-irradiation.

A technique of lens examination has been developed, which permits earlier and more accurate assay of radiation-induced opacities with low doses in mice. In particular, a difference in the time profile of "A" versus "V" opacification has been uncovered in mice exposed at 8 weeks of age. This suggests a disadvantage to combining anterior and posterior opacities to obtain a single opacification score for each lens. The incidence of "A" opacities at 20 weeks post-gamma irradiation, with a peak sensitivity at about 5 weeks (age at irradiation) bears a resemblance to that shown in the curve of radiation lethality obtained by Abrams (8) for mice irradiated at different ages up to 90 days. However, in the first experiment described in this report, lethality was evidently greatest in

the 0 and 1 week groups with loss of all animals exposed to 800 rads of Co^{60} gamma rays, while most of the animals exposed to this dose at five weeks of age survived. The acceleration of "A" opacification observed at all doses in mice irradiated at eight weeks of age is difficult to explain, unless at this age, and perhaps higher ages, the anterior lens may temporarily harbor latent damage in some way not possible at lesser ages. Lastly, a persisting lower incidence of both "A" and "V" opacities in mice irradiated at birth and one week of age appears well substantiated.

The study of lens opacification with low and divided doses of 250 kvp X-ray and neutrons of 0.43 and 1.80 Mev has yielded generally regular progression of opacity on dose. These results point rather strongly to the absence of a threshold in the initial induction of "V" opacities. Further, in the dose ranges covered, neutrons of both energies were about ten times as effective as X-rays in inducing "V" opacities, and neutrons of 0.43 Mev were slightly more effective in this regard than those of 1.80 Mev. One rad of neutrons at either energy appeared to produce definite opacification, with no evidence of the latter becoming stationary, up to 32 weeks after treatment. Thirty rads of neutrons split with a 22 hour interval showed no reduction in production of "V" opacities, whereas 975 rads of X-ray (head only) split with only a 12 hour interval showed a marked reduction in effectiveness, the latter results being comparable to Merriam's and Focht's findings in the rat with fractionated exposures to 200 kvp X-rays (9).

The presence of animal groups with small exposures was an unanticipated advantage, in that it tended to reduce observer bias. When only large doses are employed, irradiated and control animals can

be told apart easily because the radiation-induced opacities rapidly become obvious. In this circumstance, the natural tendency is for the observer to place animals with small or questionable lesions in the control animal category. In the current experiment, the observer was never certain which groups were controls, since at all times animals that received very small doses and thus had small or questionable lesions were among the groups examined.

These studies of early minimal radiation-induced opacities provide information relating to the initial damage site(s) and recovery in the lens. More complete interpretation must await completion of these experiments, histological examination, and final neutron dosimetry.

The relation of the obtained results to the cataractogenic hazard in man from exposure to ionizing radiation is not easily evaluated. It is not possible to state the degree of visual impairment which attended each of the various stages of opacification as scored in these experiments. In the opinion of the authors, central vision in the mouse probably would not be seriously impaired by a "V" opacity of less than 10%, and peripheral vision probably would not suffer from a "V" opacity of less than 30%. For any species, the degree of visual impairment for a given size opacity will be influenced by the degree to which central vision is developed in that species.

The mouse is known to be much more sensitive to radiation cataractogenesis than man, and a radiation dose which could cause a progressive opacity in the former might produce only a small stationary or transient lesion in the lens of the latter. Therefore, even if there were no threshold in man for radiation induction of initial small lens opacities,

the latter might never progress to cause a measurable loss of vision. If this is true, an "effective" threshold may well exist, below which radiation dose, progressive or significantly large stationary opacities would not occur.

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FIGURE LEGEND

- Figure 1. Opacity scoring scale employed by E. F. Riley et al.
- Figure 2. Lateral view of the facility employed in examinations of the mouse, showing the approximate position for study of the right eye. The light source, usually positioned almost parallel and adjacent to the microscope, has been omitted for clarity.
- Figure 3. Opacity scoring system used in present paper. The method of determining "percent opacification" for the individual eye is discussed further in the text.
- Figure 4. "V" opacities following exposure to gamma rays. In this and all subsequent graphs, the ordinate is given as "% opacification". This means the sum of the individual scores in percent divided by the number of eyes in each group, and not the percentage of animals with any given degree of opacification.
- Figure 5. "V" opacities for different doses at 30 weeks after exposure to gamma rays.
- Figure 6. "A" opacities at intervals after exposure to gamma rays.
- Figure 7. "A" opacities for different doses at 30 weeks after exposure to gamma rays.
- Figure 8. "V" opacities at intervals after exposure to 0.43 Mev neutrons.
- Figure 9. "V" opacities at intervals after exposure to 1.80 Mev neutrons.

Figure 10. "V" opacities at intervals after exposure to 250 kvp X-rays.

Figure 11. "V" opacities at 26 weeks after exposure to 0.43 or 1.80
Mev neutrons or 250 kvp X-rays.

Figure 12. "A" opacities at 26 weeks after exposure to 0.43 or 1.80
Mev neutrons or 250 kvp X-rays.

TABLE I

MOUSE TREATMENT GROUPSCataractogenesis Versus Age At Irradiation

Group No.	Age in weeks at irradiation	Rads of Co ⁶⁰ γ	Number of mice				Source of animals
			At irradiation	Alive at first exam.		Total	
I	less than 48 hours	0	15	8	7	15	specially bred
		500	19	10	9	19	
		600	20	6	13	19	
II	1	0	15	5	10	15	""
		500	24	12	12	24	""
		600	18	5	12	17	""
III	2	500	17	8	9	17	""
		600	23	10	13	23	""
		800	26	15	11	26	""
IV	5	0	15		14	14	regular colony ""
		500	15		15	15	
		600	15		13	13	
		800	15		15	15	
V	8	500	16		16	16	""
		600	15		14	14	""
		800	15		3	3	""

TABLE II

MOUSE TREATMENT GROUPS

Cataractogenesis with low or fractionated doses of
monoenergetic fast neutrons or X-rays. Experiment
conducted in two replicates.

Group No.	Mice/Group	Rad.	Energy Mev	Average Rad/min.	Dose in rads at			Portion Shielded
					0 hrs	12 hrs	22 hrs	
CONTROL	48	-	-	-	-	-	-	-
N-43-1	48	Neut.	0.43	0.29	1	-	-	-
N-43-2	24	Neut.	0.43	0.29	5	-	-	-
N-43-3	24	Neut.	0.43	0.29	15	-	-	-
N-43-4	24	Neut.	0.43	0.29	30	-	-	-
N-43-5	24	Neut.	0.43	0.29	15	-	15	-
N-1.8-1	48	Neut.	1.80	0.25	1	-	-	-
N-1.8-2	24	Neut.	1.80	0.25	5	-	-	-
N-1.8-3	24	Neut.	1.80	0.25	15	-	-	-
N-1.8-4	24	Neut.	1.80	0.25	30	-	-	-
N-1.8-5	24	Neut.	1.80	0.25	15	-	15	-
X-1	48	X-ray	0.250	16.9	50	-	-	-
X-2	24	X-ray	0.250	16.9	150	-	-	-
X-3	24	X-ray	0.250	16.9	350	-	-	-
X-4	24	X-ray	0.250	16.9	550	-	-	Head
X-5	24	X-ray	0.250	16.9	975	-	-	All Except Head
X-6	24	X-ray	0.250	16.9	487	487		All Except Head

STAGES OF OPACIFICATION

from: Riley, E.F., *et al*
RADIOLOGY, 67, 5: 673-84
(Ophthalmoscope)

FIGURE 1

FIGURE 2

LENS OPACITY SCORING

(BNL - SLIT LAMP)

1. "A", "V", & "P" ARE SCORED SEPARATELY.
2. AXIALLY PROJECTED AREA OF (IRREGULAR) OPACITY IS ESTIMATED BY COMPARISON TO CIRCLES AT LEFT.
3. RESULTANT PER CENT VALUE IS ADJUSTED BY MEAN OPTICAL DENSITY OF OPACITY COMPARED TO ARBITRARY STANDARD.
4. USUAL SCORING: 0, 2.5, 5, 10, 15, 20, 30, 40, 50, 75, 100 (%)

FIGURE 3

FIGURE 4

FIGURE 5

FIGURE 6

FIGURE 7

FIGURE 8

FIGURE 9

FIGURE 10

FIGURE 11

FIGURE 12