Preliminary Notes on Sensitivity of Immature Mouse Germ Cells to X-rays administered at Various Developmental Stages

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(With 4 Tables)

For estimation of genetic damage followig the exposure of human gonads to ionizing radiation, the first step is to obtain information on sensitivity of germ-cells to radiation at different stages during their development. In the mouse, this has been done by mating irradiated males to a succession of females and determining mutation rates in offspring obtained from irradiated males. It has been shown that spermatids, probably at the stage of transformation into spermatozoa, are the most sensitive to radiation, and that mature spermatozoa are relatively radio-resistant, while spermatogonia are the least sensitive (Russell 1954, Auerbach 1956, 1957, Bateman 1956a, b, 1958a, b).

The present investigation was undertaken as a study of some genetic effects of X-rays on immature germ-cells of mice at different ages.

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Material and methods: The here-described experiments were made with mice of DM/Mk strain. Males aged 7, 15, 25, 30 and 50 days were irradiated by whole body exposure at various doses of X-rays as follows: 100r, 200r, 300r and 400r. The radiation factors were :185 KVP, 6ma; filtration 0.5 mm Cu plus 0.5 mm Al; focal distance 23 cm; HVL 0.86 mm Cu, and output at the rate of 43.7 r/minute.

Each of the irradiated males was successively mated with two or three virgin females at about two-day-intervals when the males were just fifty days of age. After mating, those females were examined for the presence of vaginal plugs, a sign of successful copulation.

All females mated were subjected to examination of pregnancy rate on the basis of the number of foetuses embraced. Several females were killed at 19 days of gestation while the remaining ones were allowed to have full term of pregnancy in order that their mean litter-size might be observed.

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Results

1. *Pregnancy rates of females mated with irradiated males:* Sixty-six irradiated males were mated with 185 females. All females showed vaginal plugs, signs of copulation. Out of them, 176 females were pregnant, while 9 females were infertile. In the control group, 36 females out of 39 which mated with non-irradiated males became pregnant. The pregnancy rate of those mated females is given in Table 1. Pregnancy rate in each irradiated group showed much the same value as that in control. This observation implies that irradiation of males did not affect the pregnancy rate, as far as the doses used in this experiment are concerned.

Table 1. Rates of pregnant females mated with irradiated males

Age of males irradiated (days)	Dose (r)	No. of males irradiated	No. of mated females	No. of pregnant females	Percentage of pregnant females
7	100	4	9	6	89
	200	3	9	9	100
	300	3	10	9	90
	400	2	10	9	90
15	100	3	9	8	89
	200	3	9	9	100
	300	4	10	9	90
	400	3	9	9	100
25	100	3	9	9	100
	200	4	9	9	100
	300	3	8	9	100
	400	3	10	9	90
30	100	3	9	9	100
	200	3	9	8	100
	300	3	9	9	100
	400	4	10	9	90
50	100	4	10	9	90
	200	3	9	9	100
	300	4	10	9	90
	400	4	9	9	100
Total		66	185	176	95
Control		12	39	36	93

2. *Prenatal mortality of embryos produced by females mated with irradiated males*: Forty-two pregnant females which had mated with irradiated males were sacrificed and dissected on the 19th day after gestation. They were exmained for counts of corpora lutea, implantation and post-implantation losses. The data are given in Table 2. A total of 359 corpora lutea were observed in the 42 females.

Table 2. Embryos produced by untreated females mated with irradiated males

Age of males irradiated (days)	Dose (r)	No. of pregnant females	No. of corpora lutea	No. of implantation sites	No. of pre-implantation losses	No. of living foetuses	No. of moles
7	100	1	9	6	3	6	0
	200	3	24	18	6	15	3
	300	2	18	9	9	7	2
	400	2	19	12	7	9	3
15	100	2	18	10	8	8	2
	200	2	20	13	7	10	3
	300	3	28	18	10	16	2
	400	2	14	9	5	5	4
25	100	2	19	11	8	10	1
	200	2	15	11	4	10	0
	300	2	14	9	5	6	3
	400	2	19	11	8	8	3
30	100	3	29	19	10	19	0
	200	2	15	11	4	9	2
	300	2	18	9	9	9	0
	400	2	16	10	6	7	3
50	100	2	13	5	8	5	0
	200	2	18	13	5	13	0
	300	2	16	10	6	10	0
	400	2	17	11	6	8	3
Total		42	359	225	134	191	34
Control		7	61	37	24	36	1

Among them implanted foetuses numbered 225. A total of 191 foetuses out of those 225 implantations were normal in general appearance, and the remaining ones were moles. The irradiated groups showed an increase in number of moles, especially in the groups irradiated with high doses at comparatively younger ages.

Prenatal mortality of those foetuses is shown in Table 3. Post-implantation loss increased particularly in the group irradiated at the age of 15 days showing 13.8 per cent in frequency. That group included one female in which all embryos had died. In the group irradiated at 50 days' age, on the other hand, the post-implantation loss showed distinct decrease, being 4.7 percent in frequency.

3. *Litter-size in females mated with irradiated males*: A total of 134 pregnant females which mated with irradiated males produced 592 offspring. In control group, 147 young were obtained from 30 females mated with non-irradiated males (Table 4). Mean litter-size was found to be 4.4 in the irradiated groups, while it was 4.9 in the control. In the groups irradiated at ages of 30 and 50 days the litter-size was nearly the same as that of the control, being 4.6 and 4.7, respectively. On the other hand, the groups irradiated at ages younger than 30 days

Table 3. Prenatal mortalities of foetuses produced by untreated females mated with irradiated males

Age of males irradiated (days)	No. of pregnant females	No. of corpora lutea	% of pre-implantation losses	% of post-implantation losses	% of living foetuses
7	8	70	35.7	11.4	52.2
15	9	80	37.5	13.8	48.8
25	8	57	37.2	10.4	52.2
30	9	78	37.2	6.5	56.4
50	8	64	39.1	4.7	56.3
Total	42	359	37.3	9.7	53.2
Control	7	61	39.3	1.6	59.1

Table 4. Litter-sizes produced by untreated females mated with irradiated males

Age of males irradiated (days)	No. of pregnant females	No. of youngs	Mean litter-size
7	27	116	4.3
15	26	111	4.3
25	27	113	4.2
30	26	119	4.6
50	28	130	4.7
Total	134	592	4.4
Control	30	147	4.9

showed a litter-size at 4.2 to 4.3.

Discussion

There have been a number of investigations on the timing of various stages of spermatogenesis of mice (Leblond and Clermont 1952, Oakberg 1955, 1957). On the basis of those observations, male mice were irradiated at the following stages; spermatogonia, 7 days old; spermatocytes, 15 days old; spermatids, 25 days old, and sperm, 30 days old. Ejaculation of spermatozoa from epidermis can be found in 50 day old mice.

The pregnancy rate in each irradiated group showed much the same value as that in the control group. This fact may suggest that infertility is not due to incompetent sperm but to ill-timed mating in relation to oestrous.

Pre-implantation loss showed no difference between control and irradiated groups: this finding differs from that of Bateman (1958a). He reported that pre-implantation loss was caused by a low rate of fertilization. No sterile male was

found in the irradiated groups. It seems that irradiation of males does not affect the fertility within the doses used in this experiment.

Moles were observed in early post-implantation stages. They showed a remarkable increase in frequency in the irradiated groups, especially in the group irradiated at the age of 15 days. It seems highly probable that most of the early post-implantation loss may be due to dominant lethals.

Living foetuses were less in number in the irradiated groups than in the control. This seems to suggest an increase in number of dead embryos at early post-implantation stages. Apparently there is a parallel relation in the irradiated groups between the mean litter-size and living foetuses. The evidence presented seems to suggest that spermatids and spermatocytes are the most sensitive to X-irradiation.

Summary

Some genetic effects of X-irradiation on developmental stages of spermatogenesis were studied in females mated with irradiated males with special regard to pregnancy rate, mortality of embryos and mean litter-size. Male mice aged 7, 15, 25, 30 and 50 days were irradiated through whole body exposure with doses of 100, 200, 300 and 400r. The results obtained are as follows: 1) Pregnancy rate of females which mated with irradiated males was nearly the same as that of females mated with untreated males. 2) Moles increased remarkably in number in the irradiated groups, especially in the group irradiated at the age of 25 days, being 13.8 per cent in frequency. 3) Mean litter-size decreased slightly in the group irradiated at 25 days' age. On this basis, a possible suggestion was made that spermatocytes and spermatids are the most sensitive to X-rays.

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