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Specific-locus mutation frequencies in mouse stem-cell spermatogonia at very low radiation dose rates

(γ-irradiation/genetic radiation hazards/risk assessment/^{137}Cs)

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ABSTRACT **Experiments were undertaken to augment the information on the lowest radiation dose rates feasible for scoring transmitted induced mutations detected by the specific-locus method in the mouse. This is the type of information most suitable for estimating genetic hazards of radiation in man. The results also aid in resolving conflicting possibilities about the relationship between mutation frequency and radiation dose rate at low dose rates. There was no statistically significant difference between mutation frequencies obtained in spermatogonia with 300 R (1 R = 2.6 × 10^{-4} coulombs/kg) of γ radiation at two different dose rates, 0.005 and 0.0007 R/min, or between either of these frequencies and data obtained earlier at dose rates of 0.8 R/min and below. This supports the view in an earlier publication by one of us (W.L.R.) that, at approximately 0.8 R/min and below, mutation frequency is independent of dose rate. Because this independence is now shown to extend over the more than 1000-fold range from 0.8 to 0.0007 R/min, it seems likely that it would hold at still lower dose rates, perhaps even to the much lower dose rates encountered in most human exposures.**

There were four reasons for conducting these experiments. First, it was desirable to augment the information on mutation induction at low radiation dose rates, because this is the basis for risk estimation in humans. A second reason was to test the validity of the view (1) that, below a dose rate of about 0.8 R/min (1 R = 2.6 × 10^{-4} coulombs/min), mutation frequency in mouse spermatogonia is independent of dose rate. This had been questioned by Lyon *et al.* (2). Although not actually rejecting our view, they raised the possibility that, as dose rate decreases below about 0.03 R/min, there may be an increase in mutation frequency. Third, the results would test another opposing view, that of Newcombe (3), who, on the basis of studies on trout and *Drosophila*, suggested a possible "beneficial" effect of low levels of radiation. He supported the view, attributed to Abrahamson, that lower doses of radiation stimulate repair of part of the naturally occurring genetic damage. This would result in irradiated animals having a mutation frequency lower than the spontaneous rate in the unirradiated control. The fourth reason for these experiments was to provide material for a study by Oakberg and Palatinus (4) on survival of spermatogonial stem cells after irradiation at very low dose rates. This has a bearing on the interpretation of mutation results.

In experiments in which the radiation dose rate is so low as to require a long exposure time, the animals are necessarily exposed over a wide age range. This constitutes a difficulty when the results are to be compared with those from experiments in which the age range during exposure is much shorter. As will be shown in the next section, this factor was under better control in the current series of experiments than in earlier ones.

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A progress report on this work has appeared in abstract form (5).

MATERIALS AND METHODS

The wild-type male mice used were (101/Rl × C3H/Rl)F_1 and (C3H/Rl × 101/Rl)F_1 in approximately equal numbers. They were divided into three groups: an unexposed control group of 478, and two groups of 486 and 864 exposed to a ^{137}Cs source at distances from the source such that the radiation dose rates were 0.0007 and 0.005 R/min, respectively.

Dose rates were measured in December 1973, approximately 5 months before the start of the experiments. Two Victoreen electrometers and three chambers having ranges of 0 to 0.25 or 0 to 2.5 R were used. Instruments were calibrated by the National Bureau of Standards for ^{137}Cs. These data and earlier measurements made in 1957, 1958, 1962, 1963, and 1971 are in good agreement with the hypothesis that the exposure rate from the source was originally 17% from ^{134}Cs, with a half-life of 2.05 years, and 83% from ^{137}Cs, with a half-life of 30 years. Allowance for decay in radioactivity before and during the experiments was made in computing the dose rates given in this report.

Exposure times, adjusted to give a total dose of 300 R, were approximately 38 and 313 days for the 0.005 R/min and 0.0007 R/min groups, respectively. Exposure was continuous except for a weekly break, averaging less than 1 hr, for cage changing.

In both experimental groups, the age of the males ranged from 9 to 18 weeks at the time when exposure was started for the 0.0007 R/min group. Males in the 0.005 R/min group were divided into four subgroups. Exposure of one subgroup was started simultaneously with that of the 0.0007 R/min group, and the exposure periods of the other three subgroups were arranged to start sequentially at approximately 13-week intervals to cover the range of ages at exposure in the long exposure time of the 0.0007 R/min group. None of the experimental groups was mated until the 0.0007 R/min group had completed its exposure. The control group, whose average age was approximately 6 weeks younger than that of the experimental groups, was not mated until 6 weeks later. This was done in order to match the groups with respect to any effect that age might have on spontaneous mutation.

Exposed and control males were mated to females of our standard specific-locus test strain (T) which is homozygous for seven visible recessive marker genes (6). Each male was moved periodically back and forth between two cages, each containing one female. The offspring were scored at approximately 3 weeks of age for mutations at the seven loci. Phenotype is, by itself, almost always an accurate identification of the gene locus at which the mutation has occurred. However, breeding tests were made with all mutants, except for a few that died before

Abbreviation: R, roentgen (1 R = 2.6 × 10^{-4} coulombs/kg).

testing, to confirm their allelism. Crosses were also made to determine whether a mutation in homozygous condition was viable, sublethal (death between birth and weaning age), or lethal (death before or at birth).

Animals in subgroup 4 of the 0.005 R/min group, which were mated immediately at the end of their exposure period, produced 240 offspring from the first 2 weeks of mating and no more offspring for the 5 succeeding weeks. These 240 offspring have been excluded from the totals used in mutation rate calculation because they came from cells that were in postspermatogonial stages during the period of irradiation.

Males in the 0.0007 R/min group produced 9281 offspring from matings made in the first 7 weeks after the end of irradiation, and two of the specific-locus mutations occurred in this group. These data have not been excluded from the mutation rate calculations. Although these offspring came from cells that received part of their radiation exposure in postspermatogonial stages, the sensitivity of which to mutation induction is considerably higher than that of spermatogonia, most of the dose was received in spermatogonial stages. Furthermore, whereas inclusion of these data would be expected to bias the mutation frequency slightly in the upward direction, inclusion actually works slightly in the opposite direction.

RESULTS AND DISCUSSION

The specific-locus mutation frequencies obtained from the 0.005 R/min group are given in Table 1. There are no significant differences between the age subgroups, ranging from the youngest at the time of irradiation (subgroup 1) to the oldest (subgroup 4). There is, at most, only a slight, and statistically nonsignificant, suggestion of a trend toward a higher mutation rate in the two older subgroups combined compared with the two younger ones combined.

In Table 2, the total mutation frequency in the 0.0007 R/min group is compared with the results from the 0.005 R/min group and from the contemporary control and an earlier historical one (1). In each dose-rate group, we observed one possible additional specific-locus mutant which died shortly after birth and which was identified solely by its medium-colored eyes. In accordance with our standard procedure in earlier studies, these were not included in our computation of mutation frequencies.

Even at the lower dose rate, the mutation frequency is significantly higher than in both the contemporary and historical controls ($P = 0.009$ and 0.0003, respectively). Thus, the data do not support the view that radiation at a low dose rate might stimulate repair of spontaneous mutations.

The mutation frequencies at the two dose rates do not differ significantly from each other. The observed numbers of mutations differed by less than 2 from the numbers expected on the null hypothesis of no difference between the results at the two dose rates. The mutation frequency at 0.0007 R/min is slightly below that at 0.005 R/min. This is in the opposite direction from the results obtained earlier at 0.001 and 0.009 R/min, when the lower dose rate gave a slightly higher mutation frequency (1). Then, also, the difference was not statistically significant but, nevertheless, formed the basis for the speculation by Lyon *et al.* (2) that mutation frequency might increase as dose rate decreased in this range. Being in the opposite direction from expectation on this hypothesis, the point estimates of the current results obviously lend no support to the hypothesis.

When the current experiment was planned, it was considered possible that, if the nonsignificant difference in the earlier results at 0.001 and 0.009 R/min actually represented a true difference, the explanation for it might lie not in the suggestion made by Lyon *et al.* (2) but in the fact that the average age at exposure in the 0.001 R/min group was greater than that in the 0.009 R/min group. For example, the germ cells in older animals might be more mutable or have a lower capacity for repair. The results shown in Table 1 tend in this direction when subgroups 1 and 2 combined are compared with subgroups 3 and 4 combined. However, because the difference in mutation rates is small and not statistically significant, it is hardly worth considering as evidence. It is more cogent to point out that the design of the current experiment largely eliminated any effect of age. Furthermore, by not mating any of the groups until the 0.0007 R/min group had completed its exposure, any influence of interval between irradiation and fertilization was more equally matched in the two parts of the current experimental series. Under these better controlled conditions, there was again no significant difference between mutation frequencies at the two dose rates, and, this time, the point estimates came out with the lower frequency at the lower dose rate.

Table 3 shows the distribution of the mutations among the seven loci and their viability in homozygous condition. The two control mutations involved the *c* and *p* loci, and both were viable in homozygous condition. Only one mutation, one of the homozygous viables at the *p* locus in the 0.005 R/min groups, was intermediate between the test allele and wild-type in its phenotypic expression. The most noteworthy feature of these data is that neither the distribution of mutations among the loci nor their viability differs significantly from results obtained at higher dose rates, including those from acute irradiation at 90 R/min (7). This supports the view expressed earlier (7) that those mutations that are not repaired (the ones that still occur at low dose rates) are probably not qualitatively different from those that are repaired. This argues that what is repaired at low dose rates is simply a matter of probability, and that the dose-rate effect does not depend on a qualitative change such as, for example, a shift in the proportion of two-track and one-track events.

In using the data in Table 3 for comparisons like the comparison made in the above paragraph, it should be kept in mind that, at these levels of radiation exposure, a sizable fraction of

Table 1. Mutation frequency at seven specific loci in spermatogonia of adult mice exposed at various ages to 300 R of ^{137}Cs γ radiation at 0.005 R/min

Age group*	Offspring, no.	Mutations, no.	Frequency†
1	21,892	7	4.6
2	21,748	4	2.6
3	23,132	6	3.7
4	18,059	7	5.5

* See text for explanation.
† Mutations $\times 10^5$ per locus.

Table 2. Mutation frequency at seven specific loci in spermatogonia of mice exposed to ^{137}Cs γ radiation

Dose, R	Dose rate, R/min	Offspring, no.	Mutations, no.	Frequency*
0	Contemporary control	50,189	2	0.57
0	Historical control	531,500	28	0.75
300	0.0007	48,358	11	3.25
300	0.005	84,831	24	4.04

* Mutations $\times 10^5$ per locus.

Table 3. Distribution of mutations among the loci and viability in homozygous condition

Viability	Mutations per locus								Total
	a	*b*	*c*	*p*	*d*	*se*	*dse*	*s*	
At 0.0007 R/min									
Viable	1	1		2		1			5
Sublethal								1	1
Lethal		1		1		1		1	4
Not tested								1*	1
Total	1	2	—	3	—	2	—	3	11
At 0.005 R/min									
Viable		2	1	4				2	9
Sublethal					3				3
Lethal		1						5	6
Not tested	1	1*					1	3*	6
Total	1	4	1	4	3	—	1	10	24

* Allelism based on phenotype. Animals died before breeding tests could be made. Allelism of all other mutants was confirmed by breeding tests.

the mutations in the experimental groups may be of spontaneous origin. On the basis of the combined control groups, the number of spontaneous mutations expected in the 0.0007 R/min group is 2.5 and could be as high as 5 without exceeding the maximum likelihood one-sided 95% prediction limit. Corresponding numbers for the 0.005 R/min group are 4.4 and 8, respectively. Another point to remember in evaluating the data in Table 3 is that most of the mutants listed as "not tested" probably would have proved to be lethal in homozygous condition.

The mutation frequencies in the new data continue to support, or at least are not in conflict with, the view that radiation-induced mutation frequency in spermatogonia is independent of dose rate at dose rates below approximately 0.8 R/min. No statistically significant differences are observed between any of the present or earlier data points over the more than 1000-fold range from 0.8 to 0.0007 R/min (1). This seems to argue strongly that no further reduction in mutation frequency is likely to occur at lower dose rates, not even at the very low levels encountered in most human exposures. A companion paper (8) combines the present results with those obtained earlier for an attempted best estimate of human risks.

The data obtained by Oakberg and Palatinus (4) on spermatogonial stem-cell survival in animals from the current experiment also lend support to the validity of extrapolating the mutation frequencies to lower dose rates. At 0.005 R/min, stem-cell survival was 37% of control but, at 0.0007 R/min, the number of stem cells was unaffected. Because most of the genetic risk from radiation in man presumably involves doses or dose rates that are too low to cause spermatogonial stem-cell killing, the data in the mouse obtained at a dose rate low enough to avoid such cell killing are likely to be the most suitable for estimating human genetic hazards.

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