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Genetics

Mutation frequencies in male mice and the estimation of genetic hazards of radiation in men

(specific-locus mutations/dose-rate effect/doubling dose/risk estimation)

W. L. RUSSELL AND E. M. KELLY

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Contributed by William L. Russell, September 21, 1981

ABSTRACT **Estimation of the genetic hazards of ionizing radiation in men is based largely on the frequency of transmitted specific-locus mutations induced in mouse spermatogonial stem cells at low radiation dose rates. The publication of new data on this subject has permitted a fresh review of all the information available. The data continue to show no discrepancy from the interpretation that, although mutation frequency decreases markedly as dose rate is decreased from 90 to 0.8 R/min (1 R = 2.6 × 10^{-4} coulombs/kg) there seems to be no further change below 0.8 R/min over the range from that dose rate to 0.0007 R/min. Simple mathematical models are used to compute: (*a*) a maximum likelihood estimate of the induced mutation frequency at the low dose rates, and (*b*) a maximum likelihood estimate of the ratio of this to the mutation frequency at high dose rates in the range of 72 to 90 R/min. In the application of these results to the estimation of genetic hazards of radiation in man, the former value can be used to calculate a doubling dose—i.e., the dose of radiation that induces a mutation frequency equal to the spontaneous frequency. The doubling dose based on the low-dose-rate data compiled here is 110 R. The ratio of the mutation frequency at low dose rate to that at high dose rate is useful when it becomes necessary to extrapolate from experimental determinations, or from human data, at high dose rates to the expected risk at low dose rates. The ratio derived from the present analysis is 0.33.**

An earlier publication (1) dealt with mutation frequencies in female mice and the estimation of genetic hazards of radiation in women. The present paper provides a similar analysis for males.

One of the most important pieces of experimental information for the estimation of genetic hazards of radiation in men is the mutational response of mouse spermatogonial stem cells at low radiation dose rates. The publication of new data on this subject (2), including some at the lowest dose rate so far tested, and the inclusion of additional results in this report provide an opportunity for a fresh review of all the information now available. The data are used to compute a maximum likelihood value for induced mutation frequency at low dose rates and also to compare this with the mutation frequency at high dose rates. The results are in close agreement with earlier analyses by Russell (3) and Searle (4).

All of the data reported here were obtained by the mouse specific-locus method which uses seven visible markers and permits the detection of mutations involving any of the seven gene loci in the first-generation offspring of the irradiated parent (5). Only the results from treated and control adult males are presented here, and all the offspring came from germ cells which, at the time of irradiation, were in spermatogonial stem-cell, A_s, stage. The radiation was γ-rays (from a ^{137}Cs or ^{60}Co source) or x-rays.

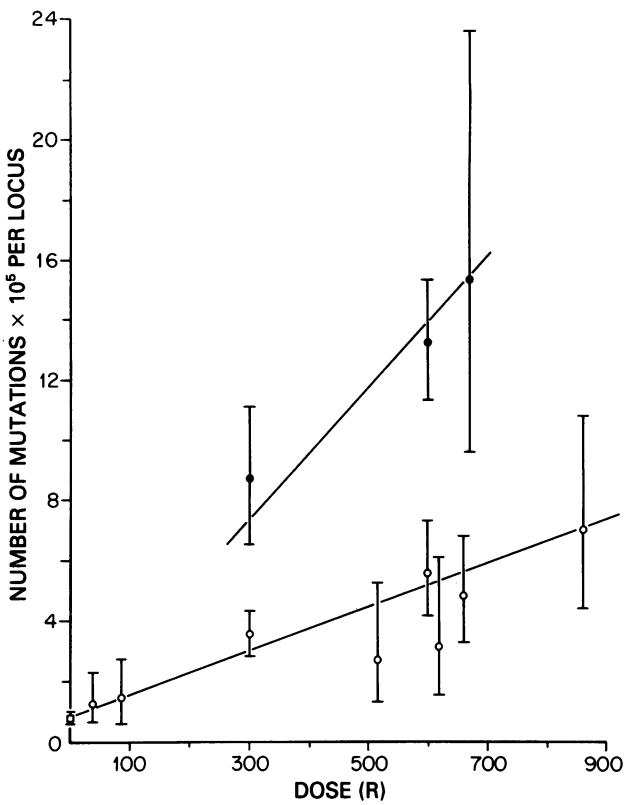

FIG. 1. Straight lines of best fit, by the method of maximum likelihood, for specific-locus data obtained for mouse spermatogonia under chronic (○) and acute (●) exposure conditions. Chronic experiments include all of those with unfractionated exposures at dose rates of 0.8 R/min and lower. Acute experiments include all of those with unfractionated exposures up to 670 R with dose rates from 72 to 90 R/min. The 90% confidence intervals of data points are shown. □, Control frequency. Data points at each dose are combined in the figure but kept separate in the computations.

The new data considered in this analysis consist of (*a*) results from exposures to 300 R of ^{137}Cs γ radiation at 0.005 R/min (1 R = 2.6 × 10^{-4} coulombs/kg) or 0.0007 R/min (2), the latter being the lowest dose rate so far tested with the specific-locus method, and (*b*) data collected some time ago, but not reported before, from the extension and repetition of an experiment reported earlier (3) in which 600 R of ^{137}Cs radiation was delivered at 0.001 R/min. These new data show no discrepancy from the interpretation (2, 3) that, although mutation frequency decreases markedly as dose rate is lowered from 90 to 0.8 R/min, there seems to be no further change below 0.8 R/min. Accordingly, for an estimate of the effect of low-dose-rate irradiation,

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

Table 1. Specific-locus mutation rate data obtained in A_s spermatogonia under chronic and acute irradiation conditions by use of the seven-locus test stock

Category	Source of radiation	Exposure, R	Dose rate, R/min	Mutations, no.	Offspring, no.	Frequency*	Ref.
Control				28	531,500	0.75	3
				11	157,421	1.00	4
				0	38,448	0	6
Chronic	^{60}Co	37.5	0.001	7	79,364	1.26	4
	^{137}Cs	86	0.001	6	59,810	1.43	3
	^{137}Cs	300	0.0007	11	48,358	3.25	2
	^{137}Cs	300	0.001	15	49,569	4.32	3
	^{137}Cs	300	0.009	10	58,457	2.44	3
	^{137}Cs	300	0.005	24	84,831	4.04	2
	^{137}Cs	516	0.009	5	26,325	2.71	3
	^{137}Cs	600	0.001	22	53,380	5.89	3†
	^{137}Cs	600	0.8	10	28,059	5.09	7
	^{60}Co	618	0.008	5	22,682	3.15	4
	^{60}Co	671 rem	0.005	20	58,795	4.86	4
	^{137}Cs	861	0.009	12	24,281	7.06	3
Acute	X-ray	300	90	40	65,548	8.72	3
	X-ray	600	90	111	119,326	13.29	3
	X-ray	670	72	12	11,138	15.39	4

* Mutations $\times 10^5$ per locus.
† And new data.

the new data and the available data from all other unfractionated radiation exposures of adult males at dose rates 0.8 R/min and below (here designated "chronic") are presented in Table 1. Within this dose-rate range the mutational response appears to be linearly related to dose (Fig. 1).

For comparison of these results with those from higher dose rates, it was decided to limit consideration of the latter to those results obtained in the range 72 to 90 R/min (here designated "acute"). It is within approximately this range that experiments on other genetic end points, such as dominant skeletal defects, are commonly performed; and the specific-locus mutation data have proved useful in providing a basis for extrapolation of skeletal and other results from high to low dose rates. Our data at 1000 R/min have not been included because they show a mutation frequency about 20% higher than that obtained at 90 R/min; this increase is not statistically significant, but results from exposed females show a similar effect.

The acute irradiation data listed in Table 1 are limited to doses between 300 and 670 R. Again, this is the range within which experiments on other genetic end points are commonly conducted. Another reason for the limitation is that, over this range of doses, the experimental points and the control mutation frequency appear to lie approximately on a straight line (Fig. 1), whereas at lower doses, judged by fractionation experiments (8), and at higher doses (9) there is marked departure from linearity.

The data were fitted to two mathematical models. The first was used to provide the best estimate of mutation frequency induced by chronic irradiation. The straight line of best fit for the chronic irradiation points and the control (i.e., not forcing the line through the control point) is described by: $Y = (8.04 \times 10^{-6} \pm 1.19 \times 10^{-6}) + (7.34 \times 10^{-8} \pm 0.83 \times 10^{-8})D$ in which Y is the mutation frequency per locus, D is dose in R, and the limits given are standard errors. The Y intercept and the slope were estimated by the method of maximum likelihood, under the assumption that the observed number of mutations in each experiment has a Poisson distribution. The result does not differ greatly from the regression formula $Y = (8.34 \times 10^{-6}) + (0.659 \times 10^{-7})D$ calculated by Searle (4) who restricted his analysis to the data then available at dose rates of 0.009 R/min and lower and who apparently used another method for determining the best fit.

The second mathematical model was used to give the best estimate of the ratio of mutation frequencies for chronic and acute irradiation over the range of acute dose rates listed in Table 1. Straight lines were fitted simultaneously through the two sets of data, again using the method of maximum likelihood. The lines were forced through the same Y intercept but not through the control point. This model is expressed as $Y_{chronic} = a + b_1D$ and $Y_{acute} = a + b_2D$. With this model, the intercept, a, was $8.10 \times 10^{-6} \pm 1.19 \times 10^{-6}$ and the slopes b_1 and b_2 for chronic and acute irradiation, respectively, were $7.32 \times 10^{-8} \pm 0.83 \times 10^{-8}$ and $2.19 \times 10^{-7} \pm 0.19 \times 10^{-7}$. These straight lines of best fit are shown in Fig. 1. The slope for chronic irradiation is almost identical with that obtained by the first model. The ratio of the slopes b_1/b_2 is 0.334 ± 0.046. This is in close agreement with our earlier estimate (3) that the slope for chronic irradiation is 31% of that for acute irradiation.

The data analyzed here can be usefully applied in three major ways for the estimation of genetic hazards of radiation in men. (The earlier analyses, which gave results closely similar to those reported here, have already been used extensively in the first two of these ways by national and international committees concerned with risk estimation.) The first application utilizes the doubling dose, the dose of radiation that induces a mutation frequency equal to the spontaneous frequency. The application implies that the doubling dose in man is likely to be similar to that in the mouse. There are, in our opinion, some important unanswered questions concerning the assumptions made in the application of the doubling-dose concept, but it is still considered useful in the latest reports by the United Nations Scientific Committee on the Effects of Atomic Radiation (10) and by the U.S. National Academy of Sciences Committee on the Biological Effects of Ionizing Radiation (11). The doubling dose computed from our best estimate of the slope of the chronic irradiation data is 110 R. This is only slightly more conservative (in the sense of predicting a greater risk per R) than the figure of 127 R derived from the earlier analysis by Searle (4). A slightly

lower doubling dose would be obtained if the analysis were restricted to the data obtained at the lowest dose rates of 0.001 and 0.0007 R. However, because there are no significant differences in mutation frequencies at dose rates of 0.8 R/min and below, it seems preferable to use the value 110 R based on the more extensive data available over this range.

The second major usefulness for risk estimation is application of the analysis to data on certain other genetic end points, when it becomes necessary to extrapolate from the experimental determinations at high dose rates to the expected risk at low dose rates. Here the ratio of mutation frequencies of chronic to acute irradiation is used. For example, it has been applied to the data on dominant skeletal defects obtained at high dose rates by Selby and Selby (12), data which became important in estimating the frequency of serious genetic disorders expected in the first-generation offspring of irradiated males (10, 11). The ratio derived from the present analysis that can be used for this purpose is 0.33.

This same ratio is used in the third application to the estimation of human risk. When the genetic damage, or an upper limit of it, can be estimated directly from human data for acute irradiation, and it becomes desirable to express this as a risk for chronic irradiation, then the ratio obtained in the mouse can be used to make the conversion. An example of this is the case in which a doubling dose estimated from a slight (although not statistically significant) genetic effect of acute irradiation from the atomic bombs is converted to an estimate of the doubling dose for human chronic exposures (13).

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