GENETIC EFFECTS OF CUMULATIVE X IRRADIATION ON THE SECONDARY SEX RATIO OF THE LABORATORY RAT[1]

G. B. HAVENSTEIN,[2] B. A. TAYLOR, JANET C. HANSEN,[3]
N. E. MORTON,[4] AND A. B. CHAPMAN

Department of Genetics, University of Wisconsin, Madison, Wisconsin 53706

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MOST estimates of the rate at which mutations are induced by ionizing radiations in mammals have been based on data involving single generations of exposure. The purpose of this report is to give the results and implications of the first 12 generations of data on the secondary sex ratio in an experiment involving the exposure of albino rats each generation, for a period of nine generations, to a standard fractionated 450r dose of X rays. A preliminary report on the design and analysis of the first four generations of the experiment has been published (CHAPMAN, HANSEN, HAVENSTEIN, and MORTON 1964).

The distribution to the offspring of radiation induced, sex-linked lethal mutations is dependent on the sex of the irradiated parent. The non-homology of the X- and Y-chromosomes and the apparent genetic inertness of the Y allow the expression of sex-linked recessive genes carried by the heterogametic sex. This difference in distribution and manifestation of sex-linked recessive genes may lead to changes in the sex ratio following exposure to mutagenic agents.

Irradiation of males only, where the male is the heterogametic sex, would be expected to cause a decrease in the number of female births since exposed males pass the irradiated X-chromosome to their daughters but not to their sons. However, completely recessive sex-linked lethals in male gametes should cause no change in the sex ratio of the first filial generation, but should cause a decrease in males in the second and later generations. If females alone are exposed, a decrease in the number of males would be expected since sex-linked recessive mutations would be expressed in sons but usually not in daughters of the exposed females.

The following assumptions are basic to the validity of the expected changes just mentioned (SCHULL and NEEL 1958). First, one must assume that although sex-limited autosomal lethal or semilethal mutations might occur, "their net effect is not such as to obscure the different effects on the sex ratio of paternal versus maternal irradiation. Clearly, were this not so, the deviations postulated could be altered in degree or direction depending upon the relative frequencies

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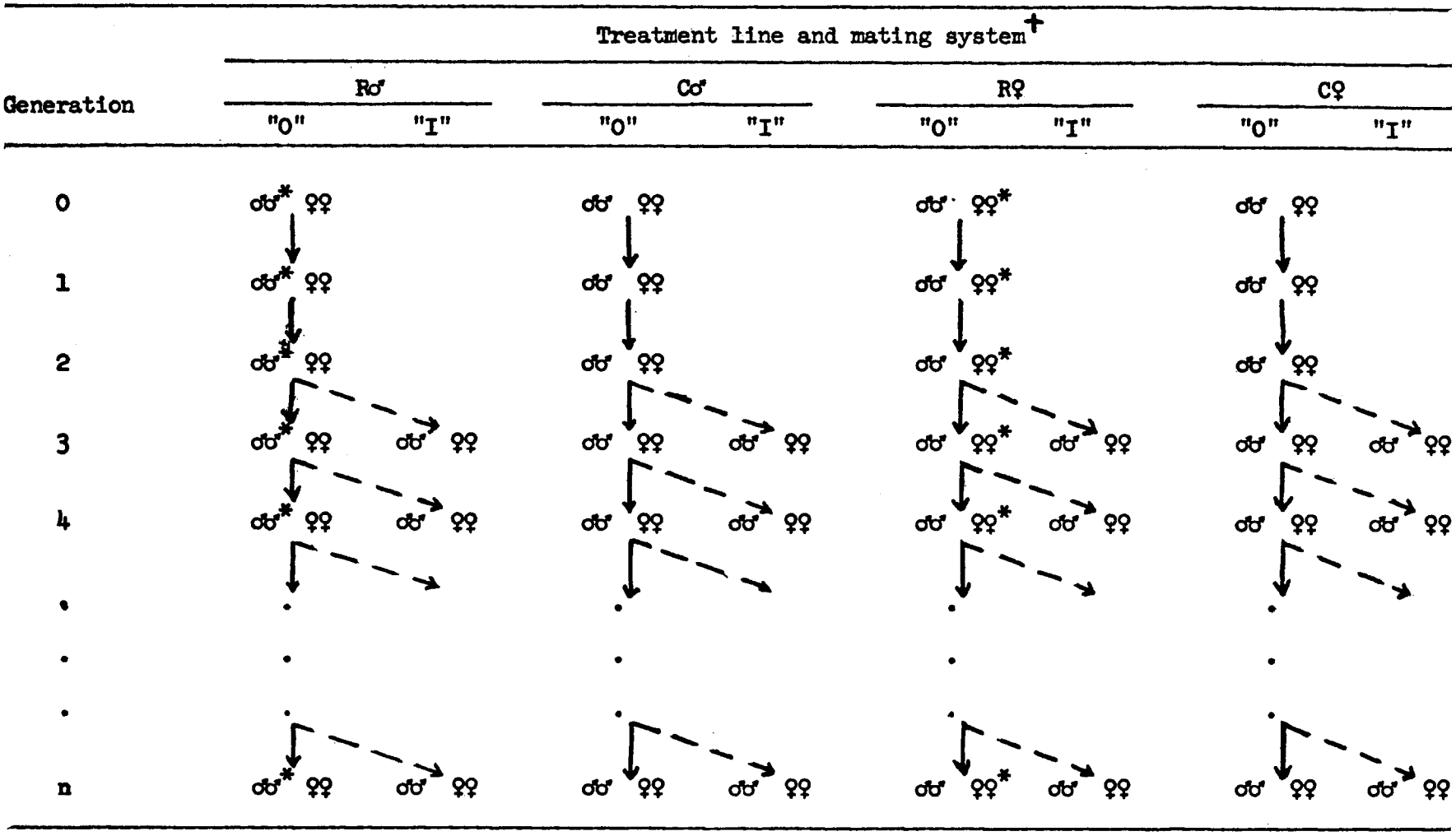
[2] Present address: Heisdorf and Nelson Farms, Inc., Redmond, Washington.

[3] Present address: 5511 Greentree Road, Bethesda, Maryland.

[4] Present address: Department of Genetics, University of Hawaii, Honolulu.

FIGURE 1.—A diagrammatic representation of the experimental design



† See text for definition of symbols.
* Exposed to 450r of X-rays.

——→ "outbred" matings, i.e. no grandparents in common.
– – → "inbred" matings, i.e. full-sibs.

of male-limited versus female-limited mutants." Second, it must be "assumed that the effect on the sex ratio of genes in the Y-chromosome is negligible," and that the X and Y chromosomes have no loci in common. The reasonableness of the former is supported by the fact that there are, at present, only a few documented cases of holandric inheritance, even though this type of inheritance should be easy to recognize (STERN 1960). In regard to the latter, LINDSLEY, EDINGTON, and VON HALLE (1960) and EDINGTON, EPLER, and REGAN (1962) reported evidence that the presence of the Y-chromosome may suppress the expression of some X-linked recessive lethals in Drosophila. If this effect is present in mammals, mutation rates calculated from sex ratio data would be underestimates in proportion to the Y-suppression effect.

MATERIALS AND METHODS

The MI4 albino rats used for the foundation stock in this experiment were descendants of SPRAGUE-DAWLEY animals which were introduced into this laboratory in May, 1940. The MI4 line is one of the highly inbred (F→1) lines which was developed in a selection experiment to show a low ovarian response to pregnant mare serum (CHAPMAN 1946; KYLE and CHAPMAN 1953; CHUNG and CHAPMAN 1958). An inbred stock was chosen to minimize the initial genetic variability and to reduce the likelihood of inbreeding depression in the progeny of sib matings.

The experimental design consists of two sub-experiments: (1) to test the genetic effects on the descendants of irradiated male ancestors, and (2) to test the genetic effects on the descendants of irradiated female ancestors. The design is illustrated by Figure 1. The symbols R♂, C♂, R♀ and C♀ represent the irradiated-male "line", its control, the irradiated-female "line" and its control, respectively. Along the side of Figure 1 are the generation numbers; the first generation in which the males or females were irradiated is designated as generation 0. Following their origin in generation 0, by random selection within the highly inbred line, the four experimental groups were maintained separately by restricted random matings. The restriction was that mates should not have any grandparents in common. The offspring from this type of mating are referred to as "outbreds" and are designated by "O" in Figure 1. The quotation marks are a reminder that this is outbreeding only with respect to the experimental population, since the base population was highly inbred. In the second and later generations (whenever a sufficient number of animals was available), a part of the matings in the irradiated and control lines was between full brothers and sisters. Offspring from this type of mating are referred to as "inbreds" and are designated as "I" in Figure 1. Following the collection of data on the "inbreds" they were discarded. The next generation was produced by "non-related" and full-sib matings from the "outbred" groups. In some generations the animals were remated. Their litters were referred to as "mating group 2."

For convenience, the experimental groups will be referred to by the following abbreviations: C♀O and C♀I, contemporary controls for the female-irradiated line produced by "outbred" and by full-sib matings ("inbred"), respectively; R♀O and R♀I, female-irradiated line "outbreds" and "inbreds," respectively; and, similarly, R♂O, R♂I, C♂O, and C♂I for the two breeding groups of the male-irradiated line and their contemporary controls.

The four lines were maintained by "outbred" matings for 13 generations, i.e., 0 through 12. In one generation in each line (generation 4 of the male-irradiated line and generation 8 of the female-irradiated line) the number of animals in the irradiated "outbred" group was reduced to such a small number, due to excessive mortality, that it was decided not to irradiate them, but to use them to expand the number of animals in the colony. Thus, during the course of the two sub-experiments nine generations of R♀O females (0 through 7 and 9) and nine generations of the R♂O males (0 through 3 and 5 through 9) were exposed to 450r of whole-body X rays.

Radiation exposure methods: Treatment consisted of 100, 150, and 200r of acute X rays

administered whole-body at 10, 12, and 14 weeks of age, respectively, to the R♂O males and R♀O females. The R♂ males and females were placed together in mating cages shortly after most of the males had reached 26 weeks of age. The R♀ males and females were placed together shortly after the last female received her final irradiation. It is assumed that these practices lead to the use of irradiated spermatogonia and dictyate oocytes for production of the progeny in the male and female irradiated lines, respectively. During the first five generations of the R♀ line (0 through 4) and for four of the first five generations of the R♂ line (0 through 3 and 5); the "outbred" animals were treated with a General Electric Maxitron resonant transformer X-ray machine (1000 Kvp, 3 ma, with HVL measure of 3.4 mm Pb). In January, 1963, a new Mattern constant potential X-ray therapy unit (250 Kvp, 15 ma, with a HVL of 2.2 mm of Cu) was installed in the Genetics Laboratory. Thereafter, beginning with generation 5 of the R♀ line and generation 6 of the R♂ line, the animals were treated with the new unit.

The X rays were administered by placing the animals in a circular lucite container (18 inches in diameter, 3 inches deep, with a top cover ¼ inch thick) consisting of 12 wedge-shaped compartments. The lucite container was placed on a turntable and rotated at 7 rpm to insure a uniform dose to all individuals. Exposure was whole body from a dorsal position. Target to source distance was 163 cm with the General Electric machine and 100 cm following the change to the Mattern unit. The Mattern machine has inherent filtration of 2.0 mm of Al, and was operated with 1.0 mm of Al and 0.75 mm of Cu additional filtration. The dose rate was approximately 18r per minute with the General Electric machine and 27r per minute with the Mattern.

THEORY AND METHODS OF ANALYSIS

Evidence, previously reported, indicates that approximately three-fourths of the mutations either induced by irradiation or occurring spontaneously and detected by the specific locus method in the mouse have been recessive lethals (RUSSELL 1965a). FRIEDMAN (1964) has shown that nearly all of the effect of mutations on mortality in Drosophila is due to lethals rather than mild detrimentals despite the fact that the latter may be much more numerous (BATEMAN 1959; BURDICK and MUKAI 1958; and MUKAI 1961 and 1964).

It was assumed for purposes of analysis that most of the radiation induced effect in the rat would also be caused by lethals, that they would be recessive with little or no selection against them as heterozygotes.

Accumulation of sex-linked recessive lethals: A part of the mutational load induced by irradiation may be due to sex-linked recessive lethals. A function of the expected rate of accumulation of this type of mutation, when irradiation occurs in only one sex and in more than one generation consecutively, may be derived in the following manner.

Assume that we are dealing with completely recessive sex-linked lethals which are therefore expressed in hemizygous males only. We will designate the first generation to receive R roentgens of irradiation as generation 0. Table 1 gives the expected rates of accumulation of this type of lethal under either male or female irradiation.

The letter m, in Table 1, reflects the mutation rate per X-chromosome per r of irradiation. Thus, mR is the mean number of induced recessive lethals per irradiated X-chromosome. In each generation of the female-irradiated line, the females receive one X-chromosome from their irradiated dam and the other from their non-irradiated sire. The males receive an X-chromosome from their irradi-

TABLE 1

Accumulation of radiation-induced recessive sex-linked lethal mutations

Generation	Average number of induced lethal mutations inherited by female	Average number of induced lethal mutations transmitted by female	Average number of induced lethals put to test in male
		Paternal irradiation	
0	$Z_0 = 0$	$T_0 = 0$	$E_0 = 0$
1	$Z_1 = \frac{mR}{2}$	$T_1 = \frac{mR}{2}$	$E_1 = 0$
2	$Z_2 = \frac{3mR}{4}$	$T_2 = \frac{3mR}{4}$	$E_2 = \frac{mR}{2}$
3	$Z_3 = \frac{7mR}{8}$	$T_3 = \frac{7mR}{8}$	$E_3 = \frac{3mR}{4}$
n	$Z_n = [1-(\frac{1}{2})^n]mR$	$T_n = [1-(\frac{1}{2})^n]mR$	$E_n = [1-(\frac{1}{2})^{n-1}]mR$
		Maternal irradiation	
0	$Z_0 = 0$	$T_0 = mR$	$E_0 = 0$
1	$Z_1 = \frac{mR}{2}$	$T_1 = mR + \frac{mR}{2}$	$E_1 = mR$
2	$Z_2 = \frac{3mR}{4}$	$T_2 = mR + \frac{3mR}{4}$	$E_2 = \frac{3mR}{2}$
3	$Z_3 = \frac{7mR}{8}$	$T_3 = mR + \frac{7mR}{8}$	$E_3 = \frac{7mR}{4}$
n	$Z_n = [1-(\frac{1}{2})^n]mR$	$T_n = mR + [1-(\frac{1}{2})^n]mR$	$E_n = [2-(\frac{1}{2})^{n-1}]mR$

ated dam and the Y-chromosome from their sire. Thus in generation 1, the females of the R♀ line carry, on the average, $mR/2$ induced recessive lethals per X-chromosome whereas the males carry mR. We assumed that the sex-linked recessive lethals would be expressed in the males, hence, those which survive to become sires would not transmit mutations of this type. Therefore, under the assumptions made, sex-linked lethals would accumulate on the maternal side only.

The expressions of interest in Table 1 are the functions $[1 - (1/2)^{n-1}]$ for male irradiation and $[2 - (1/2)^{n-1}]$ for female irradiation. These functions, X_n, were derived in a slightly different manner by CHAPMAN *et al.* (1964). They are defined as the average number of R-irradiated ($R = 450$r) X-chromosome generations exposed to test for sex-linked lethals in the male hemizygotes of generation n.

The accumulated load for sex-linked recessive lethals is independent of inbreeding.

Estimation of the rate of induction of sex-linked lethals: The number of animals observed varied from one generation and treatment group to another. Hence, the

amount of information provided by the different groups varied, and the function of the sex ratio, Y, was weighted by its invariance, I_Y, or $1/\sigma^2{}_Y$ (FISHER 1966). Iteration was used to replace I_Y with its expected value.

The following simple linear regression was used to estimate the mutation rate per chromosome per r for the trait sex ratio:

$$Y = A + MX_n$$

where: M is the mean number of induced lethals per X-chromosome, i.e., $M = mR$, and X_n is the average number of generations of R-irradiated ($R = 450$r) X-chromosomes put to test per male in generation n. M was estimated as the following weighted regression coefficient (subscripts omitted):

$$\begin{aligned} \Sigma x^2 &= \Sigma IX^2 - (\Sigma IX)^2/\Sigma I \\ \Sigma y^2 &= \Sigma IY^2 - (\Sigma IY)^2/\Sigma I \\ \Sigma xy &= \Sigma IXY - (\Sigma IX)(\Sigma IY)/\Sigma I \\ M &= \Sigma xy/\Sigma x^2 \end{aligned}$$

The dose used in this experiment, R, equaled 450r. Thus, the average number of induced lethals per chromosome per r, i.e., m, equals M divided by 450. The standard error of this estimate, assuming no error in dosage, is equal to the standard deviation of M divided by 450, i.e.,

$$\begin{aligned} m &= (1/450)M \\ \sigma^2{}_m &= (1/450)^2\sigma^2{}_M \\ \sigma_m &= \sigma_M/450 \end{aligned}$$

The relation between the observed effect on the sex ratio and the expected accumulated dose may be tested for non-linearity by:

$$\chi^2 = \Sigma y^2 - (\Sigma xy)^2/\Sigma x^2$$

The degrees of freedom equal the number of data groups minus two. A significant value for chi-square would indicate that there is significant heterogeneity between the generations as to the effect of the irradiation on the sex ratio. Thus, chi-square is a test of the goodness of fit to the linear model. If chi-square is not significant, the residual variance of the Y's around the regression line may be taken as one (MATHER 1965), i.e., the average contribution each degree of freedom is expected to make to the chi-square statistic. Therefore, if chi-square is non-significant the variance of $M = 1/\Sigma x^2$. However, if chi-square is significant it is necessary to treat chi-square as a sum of squares and divide by its degrees of freedom. The resulting mean square is the usual variance of estimate:

$$s^2{}_{y.x} = [\Sigma y^2 - (\Sigma xy)^2/\Sigma x^2]/(n-2)$$

The variance of M is then:

$$\sigma^2{}_M = s^2{}_{y.x}/\Sigma x^2$$

This regression technique was used to estimate the number of sex-linked lethals affecting the sex ratio of live young at one day of age.

Sex-linked dominant lethals would be transmitted only to the daughters of irradiated males where they would be expressed, thereby causing an increase in the sex-ratio (proportion of males). However, since they would not accumulate, their effect would be expected to be equal in all generations (except the 0 generation) and the regression estimate would be unaffected. Sex-linked dominants would not affect the sex ratio following maternal irradiation since both male and female offspring would be affected equally. Autosomal lethals should not affect this ratio since males and females should have an equal probability of receiving them and dying as a result of them. Hence the ratio would not change. Sex-influenced or sex-limited autosomal lethals could, of course, bias this estimate. Autosomal genes lethal in males only would result in a downward shift in the sex ratio and therefore an upward bias in the sex-linked lethal mutation estimate. Autosomal lethals which are expressed more frequently in females or which are restricted in their expression to females would cause an increase in the sex ratio thereby causing an underestimate or even a negative value for the sex-linked lethal rate. Completely recessive sex-influenced or sex-limited autosomal recessives would be expressed only when inbreeding occurs, thus the estimates from the "outbred" and "inbred" groups should be compared to estimate the importance of this type of mutation.

The basis for the choice of the function, Y_n, of sex ratio is given below (where a and b equal the number of males and females, respectively).

	Male	Female
Observed proportion (Gen. 0)	$\frac{a_0}{a_0 + b_0}$	$\frac{b_0}{a_0 + b_0}$
Observed proportion (Gen. n)	$\frac{a_n}{a_n + b_n}$	$\frac{b_n}{a_n + b_n}$

The expected value of the ratio, $E\left[\frac{a_n}{b_n}\right]$, in the nth generation should be proportional to the same ratio in the non-irradiated population times the probability of a male in the irradiated population receiving no induced sex-linked lethals from his female ancestor, $E\left[\frac{a_n}{b_n}\right] = \frac{a_0 e^{-MX_n}}{b_0}$. This probability, e^{-MX_n}, is the first term of the Poisson distribution (MX_n is the mean number of sex-linked recessive lethals exposed to test per male and e is the base of the natural logarithms).

Putting the above expression in the form of logarithms and rearranging:

$$E\left[\ln \frac{a_n}{b_n}\right] = E\left[\ln \frac{a_0}{b_0}\right] - MX_n$$

$$-\ln \frac{a_n}{b_n} = -\ln \frac{a_0}{b_0} + MX_n$$

$$\ln \frac{b_n}{a_n} = \ln \frac{b_0}{a_0} + MX_n$$

This expression is now in the usual regression form if we choose $Y_n = \ln \frac{b_n}{a_n}$ and $A = \ln \frac{b_0}{a_0}$. The variance of the statistic Y_n was derived as follows (where V = variance and Cov. = covariance):

$$\mathrm{V}\left[\frac{b}{a}\right] = \frac{a^2\mathrm{V}(b) + b^2\mathrm{V}(a) - 2ab\,\mathrm{Cov.}(ab)}{a^4}$$

(KEMPTHORNE 1957)

$$\mathrm{V}(a) = \mathrm{V}(b) = \left[\frac{a}{a+b}\right]\left[\frac{b}{a+b}\right](a+b) = \frac{ab}{a+b}$$

$$\mathrm{Cov.}(ab) = -\left[\frac{a}{a+b}\right]\left[\frac{b}{a+b}\right](a+b) = -\frac{ab}{a+b}$$

(MOOD and GRAYBILL 1963)

Therefore, $$\mathrm{V}\left[\frac{b}{a}\right] = \frac{b(a+b)}{a^3}$$

$$\mathrm{V}(\ln x) = \frac{1}{x^2}\mathrm{V}(x)$$

Hence, $$\mathrm{V}\left[\ln\frac{b}{a}\right] = \frac{a^2}{b^2}\left[\frac{b(a+b)}{a^3}\right] = \frac{a+b}{ab}$$

Thus, $$\mathrm{V}_{Y_n} = \mathrm{V}\left(\ln\frac{b_n}{a_n}\right) = \frac{a_n + b_n}{a_n b_n}$$

The information weight is the invariance of the statistic, Y_n, i.e.:

$$I_{Y_n} = \frac{a_n b_n}{a_n + b_n}$$

The values for Y_n, I_{Y_n}, and X_n were used to calculate the simple weighted regression mutation estimate.

Estimates of the mutation rates were also calculated for each generation separately. These "within-generation" estimates were calculated in the following manner. Let a_0 and b_0 be the numbers of live males and females, respectively, in the control population, and a_r and b_r be their counterparts in the irradiated population. If $E\left[\frac{a_r}{b_r}\right]$ is the expected value of the ratio of males to females in the irradiated group within a given generation (n), then:

$$E\left[\frac{a_r}{b_r}\right] = E\left[\frac{a_0}{b_0}\right] e^{-M_n X_n}$$

The term $e^{-M_n X_n}$ is the probability of a male in generation n not receiving a sex-linked lethal and surviving when the average number of these lethals exposed to test per male is $M_n X_n$. M_n in this case is the mean number of sex-linked lethals

which were induced per R ($R = 450$r) of irradiation and exposed to test in the males of generation n. X_n is the average cumulated number of R-irradiated generations of X-chromosomes exposed to test per male in generation n. The above equation can be rewritten as:

$$M_n = \frac{\ln \frac{b_r a_0}{b_0 a_r}}{X_n}$$

This is the maximum likelihood estimate of M_n (WOOLF 1955). The variance of the M_n estimate is:

$$V_{M_n} = \frac{\frac{1}{a_0} + \frac{1}{b_0} + \frac{1}{a_r} + \frac{1}{b_r}}{X^2_n}$$

(WOOLF 1955)

The null hypothesis is that $M_n = 0$; this may be tested by:

$$\chi^2 = \frac{M^2_n}{V_{M_n}} \qquad \text{df} = 1$$

The average number of sex-linked lethals induced per r, i.e., m, equals M_n divided by 450.

The average number of sex-linked recessive lethals induced by the irradiation over the course of the experiment can be estimated by taking a weighted average of the estimates from the different generations. That is:

$$\overline{M} = \frac{\Sigma I_n M_n}{\Sigma I_n} \qquad \text{where } I_n = \frac{1}{V_{M_n}}$$

The significance of the $\overline{M}$ estimate may be tested by χ^2 with one degree of freedom.

$$\chi^2 = \frac{(\Sigma I_n M_n)^2}{\Sigma I_n} \qquad \text{(WOOLF 1955)}$$

The estimates from the different generations may be tested for heterogeneity by:

$$\chi^2 = \Sigma I_n M^2_n - \frac{(\Sigma I_n M_n)^2}{\Sigma I_n}$$

where the degrees of freedom are one less than the number of sets of data combined (WOOLF 1955). If the heterogeneity test is not significant, the standard error of the $\overline{M}$ estimate is:

$$\text{s.e.}_{\overline{M}} = \frac{1}{\sqrt{\Sigma I_n}}$$

RESULTS AND DISCUSSION

The numbers of litters with at least one live pup at one day of age, the number of live pups at one day of age, and the percentage of males in the male-irradiated

and female-irradiated lines and in their controls are presented in Tables 2 and 3. The difference between the control and irradiated groups, within generation and mating system, in the percentages of males was not significant except in one comparison in each of the two sub-experiments—a frequency of significant differences no greater than would be expected by chance with the probability

TABLE 2

Sex ratio of live young at one day of age, classified by generation number and mating system in the control and irradiated groups of the male line

		C♂ line			R♂ line			
Generation number†	M.S.‡	Number of litters	Number of pups	Percent males	Number of litters	Number of pups	Percent males	C♂–R♂
1	O(1)§	71	400	48.3	74	447	55.3	—7.0 ± 3.4*
1	O(2)	26	145	56.6	38	199	51.3	5.3 ± 5.4
2	O(1)	118	793	51.3	140	882	47.8	3.5 ± 2.4
2	O(2)	39	190	51.6	54	348	49.4	2.2 ± 4.6
3	O(1)	103	670	45.7	131	854	49.9	—4.2 ± 2.6
3	I(1)	42	273	48.4	75	508	49.4	—1.0 ± 3.8
3	O(2)	21	152	44.7	20	121	43.8	0.9 ± 6.0
4	O(1)	72	442	52.9	62	348	47.1	5.8 ± 3.6
4	I(1)	27	203	46.8	23	103	44.7	2.1 ± 6.0
5	O(1)	107	876	50.0	75	595	48.4	1.6 ± 2.7
5	O(2)	87	657	49.5	64	478	47.1	2.4 ± 3.0
6	O(1)	123	774	49.9	118	725	49.5	0.4 ± 2.6
6	I(1)	62	392	48.5	58	318	49.1	—0.6 ± 3.8
6	O(2)	100	710	54.2	112	766	51.6	2.6 ± 2.6
7	O(1)	188	1126	48.9	144	788	48.0	0.9 ± 2.3
7	I(1)	33	181	59.1	24	124	50.8	8.3 ± 5.8
7	O(2)	88	469	52.5	68	309	51.1	1.4 ± 3.7
7	I(2)	24	130	52.3	19	107	49.5	2.8 ± 6.5
8	O(1)	111	680	48.4	107	565	50.1	—1.7 ± 2.8
8	I(1)	43	235	47.7	36	199	52.3	—4.6 ± 4.8
8	O(2)	78	449	49.2	76	411	45.3	3.9 ± 3.4
9	O(1)	131	822	51.7	85	520	48.1	3.6 ± 2.8
9	I(1)	37	250	46.8	20	97	45.4	1.4 ± 6.0
9	O(2)	152	991	48.0	121	706	48.7	—0.7 ± 2.4
10	O(1)	170	1157	50.6	131	835	48.7	1.9 ± 2.3
10	I(1)	73	362	47.5	63	302	49.3	1.8 ± 3.9
10	O(2)	151	858	51.5	115	577	49.4	2.1 ± 2.7
10	I(2)	59	314	48.7	50	228	42.1	6.6 ± 4.3
11	O(1)	72	480	48.5	75	492	46.5	2.0 ± 3.2
11	I(1)	82	523	49.3	77	474	50.6	—1.3 ± 3.2
11	O(2)	101	551	51.2	103	633	47.6	3.6 ± 2.9
11	I(2)	90	591	52.8	102	620	49.7	3.1 ± 2.9
12	O(1)	88	610	47.7	79	495	49.9	—2.2 ± 3.0
12	I(1)	66	479	50.9	59	374	51.6	—0.7 ± 3.4

† Generation number of the offspring.
‡ Mating system O = outbred; I = inbred.
§ (1) = first mating group; (2) = second mating group.
* $P < .05$.

TABLE 3

Sex ratio of live young at one day of age, classified by generation number and mating system in the control and irradiated groups of the female line

Generation number†	M.S.‡	C♀ line			R♀ line			C♀–R♀
		Number of litters	Number of pups	Percent males	Number of litters	Number of pups	Percent males	
1	O	76	564	52.7	37	153	51.0	1.7 ± 4.6
2	O	83	664	51.7	49	337	45.7	6.0 ± 3.3
3	O	89	739	47.8	70	537	47.3	0.5 ± 2.8
3	I	81	688	49.3	44	330	47.6	1.7 ± 3.1
4	O	70	525	49.5	72	530	50.6	—1.1 ± 3.1
4	I	57	452	47.6	47	322	57.5	—9.9 ± 3.7**
5	O	86	691	50.8	75	532	50.2	0.6 ± 2.9
5	I	37	279	48.0	26	167	52.1	—4.1 ± 4.6
6	O	110	838	51.0	109	736	49.5	1.5 ± 2.5
7	O	104	655	47.8	96	594	49.0	—1.2 ± 2.8
7	I	75	538	48.7	53	311	48.6	0.1 ± 3.6
8	O	133	911	49.5	92	547	50.3	—0.8 ± 2.7
9	O	228	1666	50.2	131	929	51.8	—1.6 ± 2.0
10	O	92	593	48.2	51	269	50.6	—2.4 ± 3.7
10	I	74	488	49.6	51	263	45.6	4.0 ± 3.8
11	O(1)§	77	530	49.8	54	368	50.8	—1.0 ± 3.4
11	I(2)	45	347	47.0	39	263	54.0	—7.0 ± 4.1
12	O(1)	94	594	47.5	73	469	51.8	—4.3 ± 3.1
12	I(1)	58	407	50.9	43	268	49.6	1.3 ± 3.9
12	O(2)	28	213	51.2	29	212	52.4	—1.2 ± 4.8
12	I(2)	65	511	46.2	66	503	52.1	—5.9 ± 3.1

† Generation number of the offspring.
‡ Mating system O = outbred; I = inbred.
§ (1) = first mating group; (2) = second mating group.
** $P < .01$.

levels involved. The "weighted regression" and "within-generation" methods, described previously, were used to estimate the rate at which sex-linked recessive lethals were induced by irradiation and to see if they were accumulating in an additive manner. These estimates are given in Tables 4 and 5, respectively. Estimates were calculated from the "outbred" and "inbred" data separately. The data were then combined and a single estimate was made.

The estimates calculated by the two methods are similar in magnitude. However, the "within-generation" estimates are generally lower with slightly larger standard errors than the comparable "weighted regression" estimates. Since the regression estimates were, in some cases, based on more data and since the observed effects did not deviate from the linear model (Table 4), we feel that the regression estimates should be more reliable than the "within-generation" estimates. In the calculation of the regression estimates, the observed variance was replaced by the expected variance through an iterative procedure. The weighting factors for the different groups were, therefore, probably more accurate for the "weighted regression" than for the "within-generation" estimates. The re-

TABLE 4

Weighted regression estimates of the number of sex-linked lethal mutations affecting the sex ratio at one day of age per X-chromosome per roentgen

Line	Mating system	$m \pm s_m$†	df	χ^2‡
Male	Outbred	1.88 ± 0.68**	42	38.86
	Inbred	0.81 ± 1.21	22	20.71
	Combined	1.61 ± 0.59**	66	60.21
Female	Outbred	0.10 ± 0.44	26	17.92
	Inbred	—.88 ± 0.78	12	14.57
	Combined	—.15 ± 0.38	40	34.10

† Multiply all $m \pm s_m$ values by 10^{-4}.
‡ Test of goodness of fit to the linear model.
** $P < .01$.

gression estimates, since they are slightly higher than the "within-generation" ones, also give higher confidence limits for the true mutation rates.

The non-significant tests for deviations from the linear model, Table 4, provide no evidence for the elimination of the induced lethals in any manner other than that assumed, i.e., in the male hemizygote.

The combined regression estimate from the male-irradiated line, 1.61×10^{-4}, is highly significant. The comparable estimate from the female-irradiated line, $-.15 \times 10^{-4}$, is not significant. The two estimates do not, however, differ significantly from each other (Table 4).

Most of the reported radiation induced mutation rates for mammals come from specific locus studies in the mouse and, if the present estimates are to be compared with them, an assumption must be made as to the number of lethal producing loci per chromosome. Many difficulties are inherent in this type of comparison. These arise mainly from lack of knowledge as to the number of loci involved and from our inability to determine whether mutation rates based on

TABLE 5

Weighted averages of the "within-generation" estimates of the number of sex-linked lethal mutations affecting the sex ratio at one day of age per X-chromosome per roentgen

Line	Mating system	$m \pm s_m$†	df	χ^2‡
Male	Outbred	1.51 ± 0.70*	19	12.52
	Inbred	0.89 ± 1.25	11	6.86
	Combined	1.36 ± 0.61*	31	19.57
Female	Outbred	—.08 ± 0.47	13	10.10
	Inbred	—.92 ± 0.82	6	11.74
	Combined	—.29 ± 0.41	20	22.63

† Multiply all $m \pm s_m$ values by 10^{-4}.
‡ Test for heterogeneity of the estimates from the different generations.
* $P < .05$.

the frequency of visible mutations at known loci should be compared with the incidence of lethals from a whole chromosome. The possibilities for error in comparisons of this type have been discussed by CROW (1956; 1957a, b). However, even though comparisons of mutation rate estimates from different kinds of experiments and from different species may be subject to large errors, paraphrasing the words of CROW (1957a): a poor and provisional comparison is better than no comparison at all, provided that we keep always in mind that it may be grossly in error.

As a summary of how radiation quality, dose rate, cell stage and length of time from irradiation to conception affect that rate of mutation in the mouse, data in which these variables were accurately known were taken from the literature for inclusion in Tables 6 and 7. Only those data from irradiation of mature females whose gametes were presumably in the dictyate stage of oogenesis were included in Table 6, while only estimates from spermatogonial irradiation were included in Table 7. These restrictions were made so that accurate estimates including standard errors could be calculated and so that the sex and gametic cell stage irradiated would be comparable to the present study.

The mouse rates in Table 6 were not adjusted for the spontaneous oocyte rate since no reliable estimate of the spontaneous rate exists (RUSSELL 1965a); however, all of the spermatogonial rates in Table 7 were corrected for the control rate reported by RUSSELL (1965a).

A detailed discussion of the data in Tables 6 and 7 is not warranted here. The reader is referred to the paper by RUSSELL (1965a) and to the 1966 REPORT OF THE U.N. SCIENTIFIC COMMITTEE ON THE EFFECTS OF ATOMIC RADIATION for such discussions. However, several factors which may be important to the interpretation of the present rat data are brought out in these summary tables. First, the frequency of mutation induction in the mouse is highly dependent on the dose rate. Furthermore, the dependency is not the same in oocytes and spermatogonia. Oocytes show little if any increase over controls in mutation rate with low intensity exposure, whereas, spermatogonia show a small but significant increase. At high dose rates, mouse oocytes are more mutable than are spermatogonia.

Reasoning from the above, with the present high dose rate experiment, we would have expected the oocyte rate to have been higher than the spermatogonial rate. However, (Tables 4 and 5), this was not the case. RUSSELL (1965b) has shown that the interval of time from irradiation to conception has a striking effect on the observed number of mutations from oocytes in the mouse (Table 6). Females that conceived after a seven-week interval following irradiation produced no mutant offspring, whereas, those that conceived during the first seven weeks showed a high mutation rate. In the present experiment, females in the R ♀ line were not mated until all had received three fractionated doses at 10, 12 and 14 weeks of age. Thus, the earliest the females could have conceived would have been at least four weeks following the first dose fraction. The average age at conception for the different generations, using the average age of the dams at pupping and subtracting three weeks, ranges from approximately 7 to 11 weeks following the first exposure and 3 to 7 weeks following the third exposure. So,

TABLE 6

A summary of the reported specific locus mutation rates from irradiation of female mice (dictyate oocytes), converted to a per locus per r basis

Reference	Radiation source	Dose rate r/min	Total dose (r)	Number of animals	Number of mutations	Estimated mutation rate (m) per locus per r†	95% confidence limits of m§§	
							Lower	Upper
RUSSELL 1965a	Cs^{137}	0.009	258	27,174	1	0.20	0.02	1.14
RUSSELL 1963	Cs^{137}	0.009	400	44,811§	2	0.16	0.02	0.57
RUSSELL 1963	Cs^{137}	0.009	400	7,321‖	2	0.98	0.10	3.51
CARTER 1958	Co^{60}	0.05	600	10,117	1	0.24	0.02	1.32
RUSSELL 1965b	Fission Neutrons‡	0.17	63	46,301¶	22	10.77	6.76	16.26
RUSSELL 1965b	Fission Neutrons	0.17	63	80,391††	0	0.00	0.00	1.04
RUSSELL 1963	Cs^{137}	0.8	400	61,771§	18	1.04	0.62	1.64
RUSSELL 1963	Cs^{137}	0.8	400	9,094‖	12	4.71	2.43	8.25
RUSSELL 1965b	Fission Neutrons	79.0	63	43,000¶	37	19.51	13.71	26.89
RUSSELL 1965b	Fission Neutrons	79.0	63	40,092††	0	0.00	0.00	2.09
U.N. Rept. 1966	X ray	81.0	50	127,391¶	10	2.24	1.05	4.13
U.N. Rept. 1966	X ray	81.0	50	54,621††	0	0.00	0.00	1.94
RUSSELL 1965a	X ray	90.0	200	37,297	21	4.02	2.49	6.13
RUSSELL 1965a	X ray	90.0	400	12,853	16	4.45	2.61	7.22
RUSSELL 1965a	X ray	90.0	2×200‡‡	6,086	9	5.28	2.35	10.03

† All values should be multiplied by 10^{-7}.
‡ Neutron dose in rads (gamma component is included).
§ Offspring from 2- to 4-month old nonparous females and a few from first litters of older females.
‖ Offspring from second litters of older females.
¶ Oocytes sampled up to first seven weeks after irradiation.
†† Oocytes sampled more than seven weeks after irradiation.
‡‡ Given in two 200r fractions 24 hours apart.
§§ Based on expected values of a Poisson distribution. All values should be multiplied by 10^{-7}.

TABLE 7

Some of the reported specific locus mutation rates from spermatogonial irradiation in the mouse, converted to a per locus per r basis

Reference	Radiation source	Dose rate r/min	Total dose (r)	Number of animals	Number of mutations	Mutation rate (m) per locus per r†	95% confidence limits of m§	
							Lower	Upper
RUSSELL 1965a	Cs^{137}	0.001	86	59,810	6	0.79	0.14	2.36
RUSSELL 1965a	Cs^{137}	0.001	300	49,569	15	1.19	0.62	2.07
RUSSELL 1965a	Cs^{137}	0.001	600	31,652	13	0.85	0.43	1.51
RUSSELL 1965a	Cs^{137}	0.009	300	58,457	10	0.56	0.22	1.16
RUSSELL 1965a	Cs^{137}	0.009	516	26,325	5	0.38	0.08	1.02
RUSSELL 1965a	Cs^{137}	0.009	861	24,281	12	0.73	0.36	1.32
RUSSELL 1965a	Cs^{137}	0.8	600	28,059	10	0.72	0.31	1.40
BATCHELOR *et al.* 1966	Co^{60}	0.005	609	58,795	16	0.64	0.38	1.04
PHILLIPS 1961	Co^{60}	0.007–0.009	603	10,763	2	0.44	0.04	1.58
BATCHELOR *et al.* 1966	Fission Neutrons‡	0.002–0.003	307	41,875	67	7.45	5.63	9.27
RUSSELL 1965a	Fission Neutrons	0.17	63	18,194	13	15.01	7.85	26.30
RUSSELL 1965a	Fission Neutrons	0.79	59	17,041	12	15.77	7.81	28.13
RUSSELL 1965a	Fission Neutrons	79.0	59	16,758	10	13.18	5.92	24.85
RUSSELL 1965a	X ray	9.0	600	40,326	23	1.23	0.76	1.88
PHILLIPS 1961	X ray	60–70	600	10,761	11	2.43	1.19	4.36
RUSSELL 1965a	X ray	90.0	300	65,548	40	2.66	1.86	3.66
RUSSELL 1965a	X ray	90.0	600	119,326	111	2.09	1.69	2.49
RUSSELL *et al.* 1958	X ray	90.0	1,000	31,815	23	0.96	0.60	1.45

† Corrected for control rate reported by RUSSELL (1965a). All values should be multiplied by 10^{-7}.
‡ Neutron dose in rads (gamma component is included).
§ Based on expected values of a Poisson distribution. All values should be multiplied by 10^{-7}.

if the irradiation-to-conception interval effect is similar in the rat and mouse, the small estimate obtained may be, at least in part, due to the length of time from irradiation to conception. This effect cannot be estimated from the present data. The oocyte rate obtained is probably an average from the entire post-irradiation fertile period.

Table 8 summarizes the mouse estimates most comparable to the present rat estimates. The pooled weighted regression rat estimates, Table 4, were converted to a per genome basis by multiplying them by 16. This factor comes from the averages of chromosome measurements in which the X-chromosome was found to comprise approximately one-sixteenth of the total length of the rat genome (TOBIAS 1956). The rate reported by HAVENSTEIN and CHAPMAN (1967) for the number of autosomal recessive lethals affecting the size of the litter at one day of age was multiplied by 16/15 to include the presumed contribution of the X-chromosome to the total genome rate.

The Oak Ridge estimates are given on a per locus basis so, in order to compare them with the above, they must be converted to a per genome basis. The data from RUSSELL (1965a) for the 90r per minute, 300r dose experiment (Table 7) and for the two 90r per minute, 400r total dose experiments (Table 6) are thought to be comparable in dose and method of administration to the present rat spermatogonial and oocyte data. The two 400r oocyte experiments (Table 6) did not yield significantly different results, and were, therefore, pooled, giving a value of $(4.71 \pm .94) \times 10^{-7}$ mutations per locus per r. In order to convert the mouse per locus estimates to a per genome basis we need to know the total number of lethal producing loci. No such data are available for the mouse. WALLACE

TABLE 8

Reported mouse and rat mutation rate estimates from high dose rate experiments, converted to a per genome per r basis†

Reference	Type of recessive mutation	Estimate‡	95% confidence limits‡ Lower	Upper
	Rat oocyte			
Table 4	Sex-linked lethals	—2.4	< 0	9.6
HAVENSTEIN and CHAPMAN (1968)	Autosomal lethals	1.9	< 0	4.2
	Mouse oocyte			
RUSSELL (1965a)	Autosomal visibles	7.3	4.4	10.1
	Rat spermatogonia			
Table 4	Sex-linked lethals	25.8	6.9	44.6
	Mouse spermatogonia			
RUSSELL (1965a)	Autosomal visibles	4.1	2.9	5.6
LÜNING (1964)	Autosomal lethals	0.8–2.1	< 0	6.8
LYON *et al.* (1964)	Autosomal lethals	2.6	0.6	4.6

† See text for methods of conversion to a per genome basis.
‡ Multiply each value by 10^{-4}.

(1950) has estimated the number of lethal producing loci on the second chromosome of *D. melanogaster* to be 400 (range 234–718). Thus, the number in the total genome would be expected to be around 1000. If we assume that the mouse has twice as many loci capable of mutating to lethals as Drosophila, and multiply the observed rate per locus by 2000 we would have a rough estimate of the total genome rate. However, only 77% of the observed visibles at Oak Ridge were found to be lethals when in the homozygous state (RUSSELL 1965a). On the basis of the above information, the rates given in Table 8 for RUSSELL's data are the observed rates per locus $\times$ .77 $\times$ 2000.

The estimate reported by LÜNING (1964), Table 8, is based on the ratio of embryonic deaths among the offspring of irradiated sib and non-sib crosses, corrected for the control rate, following seven consecutive generations of exposure of male mice to 276r of X rays. LYON, PHILLIPS and SEARLE (1964) irradiated hybrid F_1 males with two 600r exposures of high intensity X rays eight weeks apart. Twelve weeks after the second exposure, the males were bred to females from a different stock. F_1 males free from translocations and other factors likely to interfere with tests for recessive lethals were mated to females which were derived from a stock different from that used as F_1 females. F_2 females were then backcrossed to F_1 males, and the offspring from father-daughter matings were used to check for the presence of recessive visibles and lethals. A non-irradiated control was maintained in the same manner. The mouse has 19 pairs of autosomes. Thus, the rates reported by LÜNING and LYON *et al.* were multiplied by 20/19 to include the assumed contribution of the X-chromosome to the total genome rate (Table 8).

It should be emphasized that the conversion of RUSSELL's mouse estimate from a per locus to a per genome basis may be grossly in error. However, on the converted basis the mouse and rat oocyte estimates do not appear to be different. It should be pointed out that for estimation of the hazards of radiation to the human population, the present rat estimates and the estimates of LÜNING and LYON *et al.* may be more realistic since they are based on rates of induced lethals from an entire chromosome (X-chromosome) or sets of autosomes rather than from data on specific loci which may not be typical.

In fact, it appears that the loci which have been used in the specific locus studies of the mouse may mutate more readily than an average locus. This impression is provided by the recently reported data of LYON and MORRIS (1966) from a new set of 6 specific loci. Their average rate, although based on a limited amount of data, is approximately 4 to 5 times lower than the comparable rate from RUSSELL's data. If the loci used by LYON and MORRIS are more representative of an average locus than those used by RUSSELL and others, then RUSSELL's estimates are too high (Table 8).

LÜNING (1963) reported the sex ratio in generation four, five and six of his 276r cumulative spermatogonial irradiation experiment. There was no apparent effect of irradiation on sex ratio. The upper limit of the mutation rate to X-linked recessive lethals calculated from his data is 2.0×10^{-4}/X-chromosome/r. This encompasses the estimate made from the present experiment.

SEARLE (1964), using mice from the experiment reported by LYON *et al.* (1964), estimated the number of sex-linked recessive lethals affecting the secondary sex ratio in the mouse following high dose-rate spermatogonial irradiation. His estimate, 1.7×10^{-4}/X-chromosome/r, is almost identical to the present pooled weighted regression estimate from the male-irradiated line (Table 4). SEARLE, however, concluded, after extensive testing of the progeny from dams with aberrant sex ratios, that: "sex-linked lethals were responsible for very little, if any of the reduction in litter-size and productivity. The sex-ratio change was probably mainly a chance effect, or due to some other unknown factors; it is now clear that a much larger experiment would be needed to give a reliable estimate of the rate of induction of sex-linked lethals, preferably using sex-linked markers rather than relying on sex-ratio changes." However, based on the closeness and significance of the mouse and rat sex-ratio estimates, and on the fact that the present data did not deviate significantly from the assumed linear model, we conclude that the change in the sex-ratio is indeed real following spermatogonial exposure. We cannot, however, rule out the possibility that the effect is arising from something other than sex-linked lethals.

It seems unwise to compare the mouse and rat oocyte rates, since the effect of the time from irradiation to conception cannot be estimated from the present data.

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SUMMARY

An experiment utilizing an inbred line of rats was conducted to estimate the number of induced sex-linked recessive lethals following either maternal or paternal irradiation of ancestors. The experimental procedures and methods of analysis are described. The data provide upper 95 percent confidence limits of 0.62×10^{-4} and 2.79×10^{-4} induced recessive sex-linked lethals per irradiated X-chromosome per r of exposure for oocyte and spermatogonial irradiation, respectively. The relationship of these estimates to those from the mouse is discussed.

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