GENETIC EFFECTS OF CUMULATIVE IRRADIATION ON PRENATAL AND EARLY POSTNATAL SURVIVAL IN THE RAT

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THE objectives of this experiment are to obtain estimates of the mutational load induced by X irradiation in the rat. The traits used to reflect these mutations are sex ratio and size of litter at birth and postnatal survival.

EXPERIMENTAL PROCEDURES

The rats being used are from a highly inbred stock developed in an experiment on the effectiveness of selection for ovarian response to a gonadotrophic hormone (KYLE and CHAPMAN 1953; CHUNG and CHAPMAN 1958). The reason for using highly inbred stock was to minimize the initial genetic variability and to reduce the likelihood of inbreeding depression in the progeny of sib matings.

The basis for the experimental design and details of procedure came from theoretical predictions of the likely genetic consequences of different numbers of generations of irradiation. In addition, it was necessary to run a series of preliminary experiments to arrive at a desirable total dosage, size of each dose, rate of administration of each dose, time interval between doses, mechanics of handling rats during administration, and age of animals at exposure.

In these preliminary experiments the animals received total-body irradiation from a General Electric Maxitron resonant transformer X-ray machine (1000 kvp, HVL 3.4 mm Pb). The device for housing the animals during irradiation evolved, during these preliminary experiments, into a circular lucite container with 12 wedge-shaped compartments, each compartment large enough to accommodate, with freedom of movement, a 14-week old male rat. This container is rotated in a horizontal plane within the radiation field at 7 rev/min. All doses are measured in a position corresponding to the midline of the animal's body and are recorded as air doses. The total doses, dose fractionation, dose rate, and ages during X-ray treatment are shown in Table 1.

The attempt in these preliminary experiments was to find a treatment age and dose rate which would allow a relatively high total dose of irradiation with a minimum of somatic effects. The data collected to reflect these effects were: rate of gain and body weight at several ages, mortality rate, and reproductive performance in terms of age and rate of conception, and numbers born and weaned. These experiments also served the purpose of establishing the sterile period of the male following irradiation.

The results of these preliminary experiments led to the use in the main experiment of a total dose of 450r administered as 100, 150, and 200r at 10, 12, and 14 weeks of age, respectively. This was the largest tested dose that could be given in a practical way at a relatively young age without resulting in major somatic effects. In administering this dosage of X rays with the General Electric Maxitron 1000 kvp machine the vertical distance from the source of the X rays to the animals was 163 cm. This yielded a dose rate of about 18r per minute.

There was a reduction in the fertility of the males following the last irradiation. This resulted in complete sterility at 7 to 9 weeks after irradiation with a return to the reproductive level of the nonirradiated controls at the 12th to 13th weeks after irradiation or at about 26 weeks of age.

There are two major X-ray treated groups in the main experiment. One is a group in which

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TABLE 1

Designs of preliminary experiments

Experiment Number	Total dose, r	Dose fractionation	Approximate dose rate, r/min	Ages during treatment (weeks)
	0			
I	800	50r on alternate days	92	4–8
	0			
	800	50r on alternate days		
II	1000	62.5r on alternate days	92	4–8
	1200	75r on alternate days		
	0			
	250			
	350			
III	450	Single	92	4
	550			
	650			
	0			
	100			
IV	150	Single	18	4
	200			
	250			
	0			
	450	100, 150, 200r		
V	600	150, 200, 250r	18	10,12,14
	750	200, 250, 300r		
	0			
	450	100, 150, 200r		
VI	600	150, 200, 250r	18	4,6,8
	750	200, 250, 300r		

the males are irradiated each generation and bred to nonirradiated daughters of irradiated males. The other treatment group consists of females irradiated each generation and bred to nonirradiated sons of irradiated females.

In the male-irradiated line, males and females are placed together in mating cages shortly after most of the irradiated males have reached 26 weeks of age. It is assumed that the sperm responsible for fertilization are from irradiated spermatogonia (OAKBERG 1963). A second pairing of males and females is made in the male-irradiated line in order to produce females which are 10 to 12 weeks of age when the progeny (males) of the first mating are about 26 weeks old. This is an attempt to provide the males of each generation with females which are younger and hence likely to be more fertile than those which were born at the same time as the irradiated males.

Irradiated female rats and mice are usually fertile after low levels of irradiation but become permanently sterile after producing several litters (BEAUMONT 1962; OAKBERG 1963). Hence, in the female irradiated line the males and females are placed together in mating cages shortly after the last female receives her final irradiation at 14 weeks of age. It is assumed that the post-irradiated fertilized eggs come primarily from a population of irradiated dictyate oocytes (OHNO, KAPLAN, and KINOSITA 1960, 1961).

Two nonirradiated control groups are maintained. These are derived from the same stock as the male-irradiated and the female-irradiated groups and are bred to be contemporaneous with them. In the second and following generations part of the matings in the irradiated and control groups have been between full brothers and sisters and part between "unrelated" animals (no grandparents in common) within the inbred line. After records are taken on the inbred progeny, the animals are discarded and the experiment is continued with the descendants from the nonsib matings.

METHODS OF ANALYSIS

The methods of analysis applied to these data are patterned after the genetic load theory of MORTON, CROW, and MULLER (1956) and CROW (1958). FRIEDMAN (1964) has shown in Drosophila that nearly all the effect of induced mutation on mortality is due to lethal genes rather than to subvitals. It is assumed that this is true for rats and that the induced lethals are completely recessive and not eliminated by chance homozygosis to any appreciable extent during the early generations of irradiation.

One analysis of the survival data is by linear regression of a measure of survival (sex ratio or litter size at birth or postnatal survival) per generation on the average number of generations of chromosome irradiation exposed to test. The regression coefficient, under these assumptions, reflects the frequency of induction of lethals per generation of irradiation (450r) per chromosome.

As an example of the regression technique for estimating mutational damage, the prediction equation for postnatal survival data will be described and illustrated for two generations of nonsib mating followed by one generation of sib mating to produce "inbreds" (F = .25, within the highly inbred stock) in generation 3.

The regression equation is

$$Y_n = \overline{Y} + mR(X_n - \overline{X}) = \overline{Y} + M(X_n - \overline{X}) = A + MX_n$$

where $Y_n = \ln \frac{a_n + b_n}{b_n}$ = natural logarithm of the inverse of the proportion of survivors from birth to 69 days of age in the nth generation, where a_n = number dying in this period, b_n = number surviving in this period. The expectation is that Y_n so defined will be linearly related to X_n. Further

$\overline{Y}$ = mean of Y,

m = frequency of induction of recessive lethals per roentgen (r) per chromosome,

R = X-ray dose in roentgens per generation = 450r in this experiment,

$M = mR$ = frequency of induction of recessive lethals per R per chromosome,

$A = \overline{Y} - M\overline{X} = Y_n$ intercept at $X_n = 0$, and

X_n = average number of R-irradiated chromosomes exposed to test per individual in the nth generation (for first generation of irradiation, $n = 0$).

For outbred males (recessive sex-linked gene effects only)

$X_n = 1 - (\frac{1}{2})^{n-1}$ in male irradiated line, and

$X_n = \frac{2^n - 1}{2^{n-1}}$ in female irradiated line.

For inbred males (recessive sex-linked and autosomal gene effects)

$X_n = 1 - (\frac{1}{2})^{n-1} + 20\,(\frac{1}{4})\,\frac{n-2}{2} = 1 - (\frac{1}{2})^{n-1} + \frac{5(n-2)}{2}$ in male irradiated line, and

$X_n = \frac{2^n - 1}{2^{n-1}} + \frac{5(n-2)}{2}$ in female irradiated line.

For outbred females (recessive sex-linked and autosomal gene effects)

$X_n = 0$ in both lines.

For outbred females (dominant effects only)

$X_n = 1 - (\frac{1}{2})^n$ in both lines.

For inbred females (recessive autosomal gene effects only)

$$X_n = \frac{5(n-2)}{2} \text{ in both lines.}$$

The amount of information provided by each group of animals differs. Therefore, following FISHER's (1960) invariance weighting procedure, each statistic was weighted by the inverse of the variance of Y_n when pooling data. In the case of survivors the invariance is

$$I_n = \frac{(a_n+b_n)b_n}{a_n}.$$

Therefore (omitting subscript n),

$$\Sigma y^2 = \Sigma IY^2 - \frac{(\Sigma IY)^2}{\Sigma I},$$

$$\Sigma x^2 = \Sigma IX^2 - \frac{(\Sigma IX)^2}{\Sigma I}$$

$$\Sigma xy = \Sigma IXY - \frac{(\Sigma IX)(\Sigma IY)}{\Sigma I}, \text{ and}$$

$$M = \frac{\Sigma xy}{\Sigma x^2}.$$

The test for heterogeneity of deviations from linear regression is (MATHER 1946)

$$x^2 = \Sigma y^2 - \frac{(\Sigma xy)^2}{\Sigma x^2} \text{ with d.f.} = k-2, \text{ where } k = \text{number of groups.}$$

$$V_M = \frac{1}{\Sigma x^2} \text{ if } x^2 \text{ is not significant, and}$$

$$V_M = \frac{\Sigma y^2 - \frac{(\Sigma xy)^2}{\Sigma x^2}}{\Sigma x^2(n-2)} \text{ if } x^2 \text{ is significant.}$$

The basis for choice of the Y_n scale, $\left(\ln \frac{a_n+b_n}{b_n}\right)$ is

$$E\left[\frac{b_n}{(a_n+b_n)}\right] = \frac{b_o}{a_o+b_o} e^{-MX_n},$$

where a, b, M and X are as defined earlier, E = Expected value, and e = base of Napierian logarithms and e^{-MX_n} is the first term of a Poisson distribution or, in this case, the probability of an individual having no induced lethal mutants exposed to test per generation of irradiation (450r) in the nth group, i.e., the probability of an individual surviving when the average number of X-ray induced lethal mutants exposed to test per individual in the nth generation is MX_n.

Putting the above in terms of logarithms and rearranging,

$$\mathrm{E}\left(\ln \frac{b_n}{a_n+b_n}\right) = \ln \frac{b_o}{a_o+b_o} - MX_n, \text{ and}$$

$$-\ln\frac{b_n}{a_n+b_n} = -\ln\frac{b_o}{a_o+b_o} + MX_n .$$

Therefore

$$Y_n = \ln\frac{a_n+b_n}{b_n} = \ln\frac{a_o+b_o}{b_o} + MX_n .$$

The basis for the X_n values can be illustrated by use of Figure 1 in which nonsib matings occur in generations 0 and 1 and sib mating in generation 2. The distribution of the sex chromosomes (sex-linked genes) and one pair of autosomes (autosomal genes) can be followed through these four generations by noting that the letters designating the sex chromosomes are in the first two columns under each male and each female and those designating the autosomes in the third and fourth columns for each sex. The second and fourth columns under each sex in generations 1, 2, and 3 carry the chromosomes from the irradiated sires (♂ designated with wavy arrow), the first and third columns carry those from the nonirradiated dams (♀). The subscripts refer to the generations in which the treatment with 450r occurred. If two genes (represented by a pair of X chromosomes or a pair of autosomes) belong to the same generation of irradiation but are not identical by descent a prime appears on one letter and not the other or the same number subscript is enclosed in parentheses in one case and not the other.

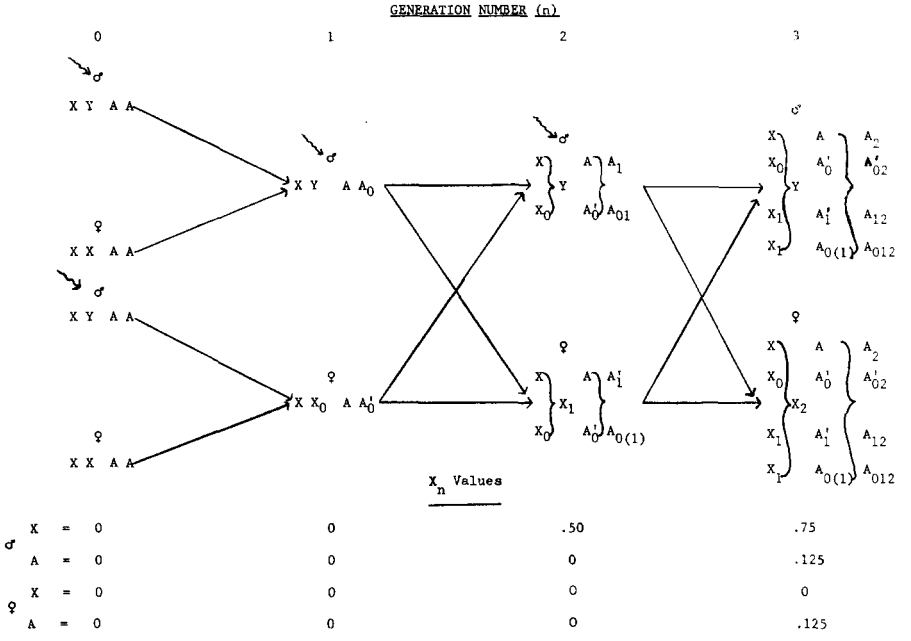

FIGURE 1.—Illustration of physical basis for estimation of average number of generations of chromosome irradiation exposed to test per individual in the nth generation (X_n)—male-irradiated line. These X_n values refer to the genes of the X chromosome and one pair of autosomes. The letters used in the above diagram and to the left of the X_n values, under the diagram, designate genes of an X chromosome or an autosome (A), or the presence of a Y chromosome. The meaning of the number subscripts of these letters is given in the text.

The inbred males of generation 3 will be used to illustrate the meaning of an X_n value which combines the X chromosome and autosome irradiation test information. It should be noted that each chromosome to the right of a bracket is expected to combine with the ones to the left with equal frequency.

Three-fourths of the males would be expected to expose irradiated X chromosomes to test in this generation. These would be X chromosomes which originated in the male shown on the third line of generation 0 and those originating in the male of generation 1. These are represented in the male of generation 3, as one X_0 and two X_1 chromosomes—probability of $\frac{3}{4} = X_n$.

The autosomes exposed to test in these males are A'_0 and A_0 (in $A'_0A'_{02}$ and $A_{0(1)}A_{012}$, respectively). These represent $1/16 + 1/16 = 1/8$ of the autosomes of this homologous pair and each is exposing to test the 450r which its ancestral chromosome received in the two irradiated males of generation 0. A comparable pattern should be found for each of the twenty autosomes. Hence, the X_n value for the autosomes of inbred males of generation 3 is $20 \times \frac{1}{8} = 2.50$.

The total X_n value (X chromosome and 20 pairs of autosomes) for inbred males of generation 3 is 3.25. This means that the number of 450r equivalent X-ray treated chromosomes (X chromosomes and autosomes) exposed to test per male in inbred generation 3 is on the average 3.25.

The functions of sex ratio and litter size at birth used for Y_n in the equations for estimating M (following the same arguments as those given for the postnatal survival estimates) are:

(a) *Sex ratio of living young at birth*

$$Y_n = \ln \frac{b_n}{a_n}.$$

b_n = number of females born alive in the nth generation.

a_n = number of males born alive in the nth generation.

$$I_n = \frac{a_n b_n}{a_n + b_n}.$$

(b) *Litter size of living young at birth*

$$Y_n = -\ln(L_n).$$

$$I_n = \frac{L^2{}_n N_n}{s^2{}_n} \text{ if } s^2{}_n \text{ homogeneous.}$$

$$I_n = \frac{L^2{}_n}{s^2{}_n} \text{ if } s^2{}_n \text{ not homogeneous.}$$

L_n = mean litter size of living young at birth in the nth generation.

N_n = number of litters in the nth generation.

$s^2{}_n$ = variance of mean litter size in the nth generation.

The statistics applied to the sex ratio and litter size data are the same as those used for the postnatal survival data.

Another basis for estimating the genetic effects of cumulative irradiation in this experiment is an intra-generation comparison of the progeny of sib mated parents within the irradiated and nonirradiated groups (Y'_{In} and Y_{In}, respectively) adjusted for the differences between the progeny of non-sib mated parents within

the irradiated and control groups (Y'_{On} and Y_{On}, respectively). These estimates are pooled over all generations.

The Y_n values used for the "within-generation" estimates are the same functions of litter size and postnatal survival as those used in the "regression method." The value of M is now estimated in terms of the average number of X-ray induced recessive lethals exposed to test per irradiation (450r) per autosome in the nth generation per individual resulting from sib mating (F = .25).

$$M = \frac{4[(Y'_{In} - Y_{In}) - (Y'_{On} - Y_{On})]}{X_n}$$

$$V_M = \frac{16\Sigma(V_{Yi})}{X^2_n}$$

Y_{On} should reflect any environmental effects identified with generation n and hence be common to Y'_{On} and Y_{On}. The difference, $(Y'_{On} - Y_{On})$, should then be a measure of sex-linked effects of irradiation. It should also reflect any dominant genetic effects of irradiation expressed in the individual in the nth generation or expressed as dominant maternal effects. In addition, this difference may reflect a somatic effect of irradiation on the maternal phenotype in the female irradiated line. In both lines, a difference between these two Y's may result from selective elimination of the irradiated parent due to somatic effects of irradiation.

The difference, $(Y'_{In} - Y_{In})$, should reflect the same effects as $(Y'_{On} - Y_{On})$ and, in addition, induced recessive lethals may be made homozygous by the inbreeding (F = .25). Adjustment for any inbreeding effect independent of irradiation and for environment is accomplished by subtracting Y_{In} from Y'_{In}. The difference between these two differences should represent an estimate of one-fourth (F = .25) of the average number of X-ray induced recessive lethals per individual per irradiation (450r). This value multiplied by 4 and divided by X_n (the average number of generations of chromosome irradiation—450r each—exposed to test per individual in the nth generation) should give an estimate of M, the average number of recessive lethals induced per 450r per generation per autosome. Each estimate of M was weighted by the inverse of its variance. These were pooled to provide an average M.

The "within-generation" method of estimating the number of lethals induced per X chromosome per R (450r) based on the sex-ratio data is

$$M = \frac{\ln \dfrac{b_r a_o}{b_o a_r}}{X_n}, \text{ and}$$

$$V_M = \frac{\dfrac{1}{a_o} + \dfrac{1}{b_o} + \dfrac{1}{a_r} + \dfrac{1}{b_r}}{X^2_n}$$

where a_o, b_o = number of control males and females respectively, and a_r, b_r, = number of irradiated males and females respectively. This is the maximum likelihood estimate (Woolf 1955; Fisher 1960).

Calculations, based on estimates of sex-linked recessive mutation rate in mice

TABLE 2

Sex ratios at birth in control and irradiated groups of male and female lines

Generation No.	Mating system†	Male line							Female line						
		Control (C)			Irradiated (R)				Control (C)			Irradiated (R)			
		No. litters	No. rats	Percent males	No. litters	No. rats	Percent males	C—R	No. litters	No. rats	Percent males	No. litters	No. rats	Percent males	C—R
1	O	96	545	50.5	109	646	54.0	—3.5	76	564	52.7	37	153	51.0	1.7
2	O	155	983	51.4	194	1230	48.3	3.1	83	664	51.7	49	337	45.7	6.0
3	O	124	822	45.5	151	975	49.1	—3.6	89	739	47.8	70	538	47.3	0.5
3	I	42	273	48.4	75	508	49.4	—1.0	82	698	49.3	44	330	47.6	1.7
4	O	72	442	52.9	62	348	47.1	5.8	70	525	49.5	72	530	50.6	—1.1
4	I	27	203	46.8	23	103	44.7	2.1	57	452	47.6	47	322	57.5	—9.9**
Total		516	3268	49.4	614	3810	49.4	0	457	3642	49.8	319	2210	49.6	0.2

† O="Outbred". I="Inbred" (result of sib mating).
** $P<.01$.

and man, led to the expectation that at least nine generations of irradiation with the dosage used here would be necessary to give sufficient power to the tests to detect a mutation effect. Hence, the results presented herewith (four generations analyzed) must be looked on as preliminary estimates.

RESULTS AND DISCUSSION

Tables 2, 3, and 4 give the mean values for sex ratio, litter size, and postnatal survival classified by irradiation line (male and female), by generation number, by mating system, and by treatment group (control and irradiated). The differences between the control and irradiated group means ($C-R$) are shown in these tables. The concrete partial regression coefficients of litter size on treatment group, holding age of dam and age of dam squared and cubed constant, are also given in Table 3.

TABLE 3

Numbers born alive in control and irradiated groups of male and female lines

Generation Number	Mating system†	Male line				Female line			
		Control C	Irradiated R	C—R		Control C	Irradiated R	C—R	
				Unadj.	Adj.‡			Unadj.	Adj.‡
1	O	5.6	6.0	—0.4	0.5	7.4	4.1	3.3	4.0**
2	O	6.7	6.3	0.4	0.8*	8.0	6.9	1.1	1.1*
3	O	6.5	6.5	0.0	0.3	8.3	7.7	0.6	0.7
3	I	6.5	6.8	—0.3	0.3	8.5	7.5	1.0	1.0*
4	O	6.1	5.6	0.5	0.2	7.5	7.4	0.1	0.4
4	I	7.5	4.5	3.0	2.5**	7.9	6.9	1.0	1.6

The values in body of the table are numbers born alive.
† O="Outbred". I="Inbred" (result of full sib-mating).
‡ Adjusted for: age of dam, (age of dam)^2, (age of dam)^3.
* $P<.05$. ** $P<.01$.

TABLE 4

Percent survival birth to 69 days in control and irradiated groups of male and female lines

Generation Number	Mating system	Male line			Female line		
		Control C	Irradiated R	C—R	Control C	Irradiated R	C—R
Males							
1	O	92.7	88.8	3.9	95.8	97.3	—1.5
2	O	88.4	85.4	3.0	89.5	90.9	—1.4
3	O	90.0	88.1	1.9	77.6	74.4	3.2
3	I	91.7	90.7	1.0	76.3	77.7	—1.4
4	O	70.5	69.6	0.9	76.1	74.3	1.8
4	I	67.4	71.1	—3.7	78.8	71.0	7.8
Females							
1	O	89.3	92.2	—2.9	95.4	91.9	3.5
2	O	87.9	85.1	2.8	90.0	86.9	3.1
3	O	90.5	93.6	3.1	73.7	80.3	—6.6
3	I	91.4	90.6	0.8	76.8	82.0	—5.2
4	O	64.1	71.7	—7.6	75.1	74.8	0.3
4	I	71.3	66.7	4.6	79.5	79.6	—0.1

Estimates of the rate of induction of lethals per r of X rays per chromosome in the male and female irradiated lines are given in Tables 5 and 6, respectively. These estimates, with their standard errors, are classified by the trait used in the estimate and by the method of estimation. The last column of each table gives the basic assumptions used in assigning values to X_n and hence in estimating m.

It is clear from the ratios of these values of m to their standard errors that these first four generations of irradiation do not provide clear evidence of induction of

TABLE 5

Estimates of rate of induction of lethal mutations per roentgen of X-ray per chromosome in male irradiated line

Classification	$m \pm s_m$* "Regression"	$m \pm s_m$* "Within"	Basic assumptions
Sex ratio (birth)	2.14 ± 1.5	0.86 ± 1.7	Sex-linked recessives
Litter size (birth)		0.05 ± 0.03	Autosomal recessives
Outbred	—.56 ± 3.1		Sex-linked recessives
Inbred	1.20 ± 0.8		Sex-linked and autosomal recessives
Survival (birth to 69 days)			
Males		—.27 ± 0.3	Autosomal recessives
Outbred	1.28 ± 1.7		Sex-linked recessives
Inbred	—.10 ± 1.0		Sex-linked and autosomal recessives
Females		0.54 ± 0.3	Autosomal recessives
Outbred	—.62 ± 1.7		Sex-linked and autosomal dominants
Inbred	0.18 ± 1.2		Autosomal recessives

* All values multiplied by 10^{-4}.

TABLE 6

Estimates of rate of induction of lethal mutations per roentgen of X-ray per chromosome in female irradiated line

Classification	$m \pm s_m$* "Regression"	$m \pm s_m$* "Within"	Basic assumptions
Sex ratio (birth)	—.05 ± 0.7	—.20 ± 0.7	Sex-linked recessives
Litter size (birth)		0.53 ± 0.1	Autosomal recessives
Survival (birth to 69 days)			
Males		—.14 ± 4.6	Autosomal recessives
Outbred	0.99 ± 1.1		Sex-linked recessives
Inbred	0.21 ± 0.2		Sex-linked and autosomal recessives
Females		0.10 ± 4.3	Autosomal recessives
Outbred	0.83 ± 2.2		Sex-linked and autosomal dominants
Inbred	—.02 ± 0.1		Autosomal recessives

* All values multiplied by 10^{-4}.

sex-linked or autosomal, dominant or recessive, lethals. Some of the values found for rate of induction of sex-linked and autosomal recessive lethal mutations (m) are about 1½ to 5 times their standard errors and may be considered suggestive of an effect. These are the estimates based on litter size in the female line ($m = 0.52 \times 10^{-4}$), litter size in the male line ($m = 0.05 \times 10^{-4}$ and 1.20×10^{-4}), survival in the male line ($m = 0.54 \times 10^{-4}$) and sex ratio in the male line ($m = 2.14 \times 10^{-4}$). This last estimate of rate of induction of sex-linked lethals per r based on sex ratio data in the male irradiated line is not inconsistent with values which may be derived from data on irradiation-induced mutants in man and mice.

TURPIN, LEJEUNE, and RETHORE (1956) reported that the sex ratio of the children of women irradiated for treatment of defects, which did not include cancer, was 0.46 ± 0.04. The number of induced lethals can be estimated from the equation for the "within-generation" method of analysis of sex ratio data using 0.51 as the normal sex ratio. It is assumed that the gonadal irradiation of these women was approximately 450r. This yields an estimate of 4.16×10^{-4} lethal mutations per r per sex chromosome.

The data of NEEL and SCHULL (1956) and SCHULL and NEEL (1958) on the progeny of survivors of the atomic bombs at Hiroshima and Nagasaki permit the same kind of estimate. It is estimated that the frequency of males was reduced —0.55 percent per 100r of maternal irradiation. This value yields 2.2×10^{-4} as the estimate of lethal mutations per r per sex chromosome.

RUSSELL's (1951) mouse data provide an estimate of 2.5×10^{-7} per gene per r. On the assumption of 500 loci on the X chromosome, the lethal mutation rate per r per sex chromosome in mice would be estimated to be 1.25×10^{-4}.

It should be borne in mind that the estimates of rate of induction of lethals made in this experiment are based on the assumptions that these mutations are completely recessive, fully lethal in homozygotes and hemizygotes, that epistasis does not play a role in their expression, and that chance homozygosis is negligible.

If some of the lethals are partially dominant it would be expected that their true frequency would be underestimated by the methods used. This would result from elimination of a part of the lethal genes in the heterozygote and hence a lowered frequency of those assumed to be exposed to test in subsequent generations.

If some of the induced lethal mutations have a heterotic effect, the estimates made by the methods used would overestimate their true mutation rate. The genetic load exposed to test would be partly mutational and partly segregational. The value used for the number of irradiated chromosomes exposed to test per individual is based on the assumption that the heterozygote for a mutant is equal in fitness to the homozygous dominant.

If the lethals are not inherited independently, then a chromosome which carries more than one lethal when it is exposed to test will, of course, be counted as one lethal. This would result in the true frequency being underestimated by the methods used. The probability of linkage between two induced mutants would seem to be low enough to allow it to be ignored as a basis for underestimate.

It would appear that epistasis may be responsible for leading to either an

underestimate or overestimate of the rate of induction of lethals. There is such limited information on the kind and extent of such nonadditive gene action that there is little basis for speculation. It would, however, appear unlikely that nonallelic gene interaction would play an important role because of the infrequency with which two or more induced mutants are simultaneously exposed to test.

The induction of Y-suppressed sex-linked lethals (LINDSLEY, EDINGTON and VON HALLE 1960) would be expected to result in an underestimate of the number of radiation-induced sex-linked lethals estimated from male mortality data. These mutants would, however add to the number of induced lethals exposed to test in inbred females and be interpreted as autosomal mutants, thereby causing an overestimate of these in inbred females. They would also gradually accumulate and be exposed as homozygotes in "outbred" females. This would appear to be a minor basis for error of estimation.

If some of the sex-linked induced mutants are subvitals instead of lethals as assumed here, these methods would yield an overestimate of m. If autosomal subvitals are induced when the estimates are predicated on induction of autosomal lethals, the estimates of m would be valid.

In all these estimates of frequency of induction of lethal mutations, linearity of rate of induction in relation to dose has been assumed in calculating rate per r. RUSSELL (1956, 1962) and RUSSELL, RUSSELL, and KELLY (1958) found nonlinearity in this relationship for induced spermatogonial mutation rate in certain dose ranges in mice. OFTEDAL (1964) and MULLER, HERSKOWITZ, ABRAHAMSON, and OSTER (1954) found a similar association in Drosophila. In other dose ranges the mutation rates for recessive sex-linked lethals induced by X irradiation of spermatogonia in Drosophila were found to be proportional to dose (FRIEDMAN 1964; ABRAHAMSON and FRIEDMAN 1964; IVES 1959). Except for OFTEDAL (1964) these departures from linearity of mutation rate on dose occurred at high doses. It is not known whether nonlinearity applies to the range from 1r to 450r in rats.

The experiment described herewith is being continued until at least nine generations of irradiation have been tested for rate of induction of lethal mutations and for the genetic effects on body weights at different ages and on age at opening the vagina.

At the termination of the present phase of the experiment, additional research should be undertaken in an attempt to estimate the extent to which partial dominance, overdominance, sex-limited expression, maternal effects, etc., need to be considered in interpreting the results from the main experiment.

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SUMMARY

Experimental procedures, methods of statistical analyses, and results of the first four generations of an experiment to estimate the genetic effects induced by X irradiation in the laboratory rat are presented. The traits used to reflect these effects are sex ratio, number of live births per litter, and postnatal survival.

No clear evidence of genetic effects of this cumulative irradiation is available from the results in the first four generations of irradiation. One estimate which is suggestive of an effect is based on the sex ratio data in the line in which male ancestors received 450r of X irradiation each generation. In this case, it is estimated that the rate of induction of sex-linked recessive lethals per r is $(2.14 \pm 1.5) \times 10^{-4}$. This estimate is not inconsistent with those made in humans and mice. (Two estimates of the rate of induction of sex-linked recessive lethals per r in man are 2.2×10^{-4} and 4.2×10^{-4}. An estimate of the rate of induction per r for sex-linked recessive lethals in mice is 1.25×10^{-4}.)

The estimates of rate of induction of lethals made in the present experiment are based on the assumption that these mutations are completely recessive, fully lethal when homozygous or hemizygous, that epistasis does not play a role in their expressions, and that chance homozygosis is negligible. The effect on the estimates of rate of induction if these assumptions are not correct is discussed.

On the basis of estimates of sex-linked mutation rate in humans and mice, it is predicted that at least nine generations of X irradiation with the dosage used here will be needed for a trait such as sex ratio to provide sufficient power to the tests to detect a mutation effect. The experiment will be continued to at least this stage of development.

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