THE EFFECT OF PRE-FERTILIZATION MATERNAL IRRADIATION ON PRENATAL, PERINATAL AND POSTNATAL SURVIVAL IN THE ALBINO RAT[1]

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THE objective of this experiment was to estimate the number of genetic deaths resulting from pre-fertilization whole-body exposure of female albino rats to a 450r dose of X radiation each generation for 9 generations followed by several generations without irradiation. The numbers of live and dead young per litter at birth and postnatal survival were used to reflect the number of induced autosomal recessive lethals.

EXPERIMENTAL PROCEDURES

The rats used were from a highly inbred stock (F→1) developed in a selection experiment for high and low ovarian response to a gonadotrophic hormone (KYLE and CHAPMAN 1953; CHUNG and CHAPMAN 1958). Two "lines", designated as control (C) and irradiated (R), were formed by random selection within litters of the inbred stock. These "lines" were maintained for 13 generations (0 through 12) by restricted random matings, the restriction being that the mated individuals should have no grandparents in common. The offspring from these matings are referred to as "outbreds". The quotation marks refer to the fact that the outbreeding was within the highly inbred (F→1) stock. Part of the animals in generations 3, 4, 5, 7, 10 and 12 were produced by full-sib matings and are referred to as "inbreds". They were not irradiated or allowed to reproduce. The design and mating procedure is as illustrated in Figure 1 of HAVENSTEIN *et al.* (1968).

Full-sib mating pairs were selected at random from the "outbred" litters of the previous generation. Because the litters from which they came had to contain at least one male and one female, individuals from small litters and litters with no males or no females were eliminated from being parents of the "inbred" group. This may have resulted in some bias in the results if the number of induced mutations which was put to test by the full-sib matings was dependent on the size of the litter from which the sib pairs were obtained.

The "outbred" females of the irradiated group were exposed to 100, 150 and 200r doses of whole-body X rays at 10, 12 and 14 weeks of age, respectively, in generations 0 through 7 and in generation 9. In some generations the animals were remated. Their litters were referred to as "mating group (2)". Further details of the experimental design and procedures have been published by CHAPMAN *et al.* (1964) and by HAVENSTEIN *et al.* (1968).

THEORY AND METHODS OF ANALYSIS

For analysis, it was assumed that most of the mutations induced in these rats

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would be recessive lethals, that they would have little or no selection against them in the heterozygous state, and that they would not be eliminated to any appreciable extent by chance homozygosity during the course of the experiment (HAVENSTEIN *et al.* 1968).

One component of the total induced genetic load is associated with the accumulation of autosomal recessive lethals. Since any autosomal mutation that occurs in an oocyte of an irradiated female will be passed to only one of her immediate progeny, a two generation lag must occur before inbreeding can be used to determine their frequency. The number of autosomal recessives expressed is a function of the amount of inbreeding practiced.

The rate of accumulation of autosomal recessive lethals when the female parent only is irradiated can be derived in the following manner. Assume complete lethality when a mutation becomes homozygous as a result of inbreeding. If generation 0 is the first generation of females to receive R roentgens of irradiation, ($R_0 = R_1 = R_2 = \cdots = R_n$) and m equals the average number of completely recessive autosomal lethals induced per r of exposure per autosomal set (haploid), then mR newly induced autosomal recessive lethal mutants would be carried, on the average, in each female gamete per generation of irradiation. When the dam only is irradiated, she transmits these mR mutations from her own exposure plus the average number of mutations she inherited from her parents. The nonirradiated males transmit only the ones they inherited. The expected rate of accumulation of this type of lethal is presented in Table 1.

Estimates of the number of autosomal recessive lethals affecting the number of animals per litter were made as follows. Let Y_n be a function of the litter size

TABLE 1

Accumulation of autosomal recessive lethals in "outbred" males and females per set of autosomes

Generation number	Average number of induced lethals per autosomal set: From dam	From sire	In offspring
0	0	0	0
1	mR	0	$\frac{mR}{2}$
2	$mR + \frac{mR}{2}$	$\frac{mR}{2}$	$\frac{2mR}{2}$
3	$mR + \frac{2mR}{2}$	$\frac{2mR}{2}$	$\frac{3mR}{2}$
4	$mR + \frac{3mR}{2}$	$\frac{3mR}{2}$	$\frac{4mR}{2}$
n	$mR + \frac{(n-1)mR}{2}$	$\frac{(n-1)mR}{2}$	$\frac{nmR}{2}$

and $E[L_n]$ equal the expected value for the mean litter size in generation n. If one assumes a linear model for the accumulation of induced autosomal recessive lethals with generation, then:

$$E[L_n] = E[L_0]\ e^{-MX_n} \qquad (1)$$

where L_0 is the mean number per litter for the control group or base population (Generation 0) and e^{-MX_n} is the first term of the Poisson distribution and equals the probability of an individual receiving no induced autosomal recessive lethals and surviving when the number of these lethals exposed to test per individual is MX_n. M is equal to mR, i.e., the number of mutations induced per 450r exposure. X_n is the average number of generations that the sets of autosomes, put to test in generation n, had been exposed to 450r of irradiation. Accumulation of autosomal recessive lethals per gamete occurs at a rate proportional to $n/2$ (Table 1); however, the number put to test by inbreeding in generation n is the number that had accumulated two generations previously multiplied by the inbreeding coefficient (F), thus, $X_n = F(n-2)/2$. Putting equation (1) in the form logarithms and rearranging yields equation (2):

$$-\ln(L_n) = \ln(L_0) + MX_n \qquad (2)$$

Thus, the function of litter size used for analysis, i.e., Y_n, is the negative of the natural logarithm of the mean number per litter.

Generations 3, 4, 5, 7, 10 and 12 consisted of four progeny groups each, i.e., control "outbreds" and "inbreds" and irradiated "outbreds" and "inbreds". Let Y_{CO_n}, Y_{CI_n}; Y_{RO_n}, and Y_{RI_n} represent the Y_n values from these four progeny groups in generation n, respectively.

Since the expression for Y_n is the negative of the natural logarithm of the average number per litter ($-\ln L_n$) the expectation, if inbreeding brings induced autosomal recessive lethals to expression, is that $Y_{RI_n} >> Y_{CI_n}$ and $Y_{RO_n} > Y_{CO_n}$.

Y_{O_n} should reflect any environmental effects identified with generation n and should hence be common to both Y_{RO_n} and Y_{CO_n}. The difference, $(Y_{RO_n} - Y_{CO_n})$, should reflect any somatic effects of the irradiation in the dam which are expressed as maternal effects in the offspring. In addition, this difference should reflect any recessive or dominant sex-linked effects of the irradiation and any dominant genetic effects which are expressed directly in the individual of the nth generation or as dominant maternal effects. A difference between these two Y's might also result from selective elimination of the irradiated parents due to the somatic effects of the irradiation.

The difference, $(Y_{RI_n} - Y_{CI_n})$, should reflect the same effects as $(Y_{RO_n} - Y_{CO_n})$ and, in addition, the effects of induced autosomal recessive lethals made homozygous by the inbreeding ($F = .25$). This difference should also adjust for any inbreeding effect independent of irradiation and for environmental effects common only to the "inbreds". The difference between these two differences should on the average represent the effect of one-fourth ($F = .25$) of the number of induced recessive autosomal lethals per irradiation (450r). This difference divided by X_n (the average cumulated number of 450r-irradiated sets of autosomes exposed to test per individual in generation n) should provide an estimate of M_n, i.e., the average number of recessive lethals induced per R and present in a set of autosomes in generation n. Hence, M_n, may be estimated by:

$$M_n = \frac{[(Y_{\mathrm{RI}_n} - Y_{\mathrm{CI}_n}) - (Y_{\mathrm{RO}_n} - Y_{\mathrm{CO}_n})]}{X_n} \quad (3)$$

where $X_n = F(n-2)/2$

The variance of this estimate is:

$$V_{M_n} = \frac{(V_{\mathrm{RI}_n} + V_{\mathrm{CI}_n} + V_{\mathrm{RO}_n} + V_{\mathrm{CO}_n})}{X^2_n} \quad (4)$$

The variances, s^2_n, of the litter size, L_n, of the four data groups within a generation were tested by the method of BARTLETT (SNEDECOR 1956) and were found not to be heterogeneous. Thus, the variance of Y_n for the various data groups, V_{Y_n}, is:

$$V_{Y_n} = \frac{s^2_n}{L^2_n N_n} \qquad \text{(FISHER 1966)} \quad (5)$$

where N_n is the number of litters per group.

The estimates from the different generations were tested for heterogeneity and pooled by the method described by HAVENSTEIN *et al.* (1968).

The average number of autosomal recessive lethals induced per irradiated set of autosomes per r, i.e., m, equals M_n divided by 450, and the standard deviation of m equals the standard deviation of M_n divided by 450.

RESULTS

The number of live and dead animals per litter were recorded between 24 to 48 hours following birth. The size of the litter at birth is partially dependent on the age of the dam at parturition. In these data this effect had both linear and quadratic components, i.e., litter size changed with advancing age in a curvilinear fashion—the youngest and oldest dams tending to have smaller than average litters.

Since the average age of the dams for the four breeding groups varied within some generations, all of the data were adjusted for this trait and for age of dam squared. The adjustment was made by use of a multiple regression of the number in the litter on radiation group (controls coded 1 and irradiated 0), on age of dam and on age of dam squared. These adjustments were made within generation and mating system.

The number of litters, the adjusted means, the differences between the control and irradiated groups and the standard errors of these differences are presented in Tables 2 and 3 for number alive and for number dead per litter at one day of age.

The generations in which the females were irradiated (1 through 8 and 10) show that large significant differences occurred between several of the irradiated groups and their controls in the total number of live young, in the number of live males and in the number of live females at one day of age. However, RUSSELL and RUSSELL (1954) have shown that the number of live young per litter is not

TABLE 2

Adjusted means of the total number of live young, the number of live males and the number of live females per litter in the control (C) and irradiated (R) groups

		Total number of live young			Number of live males			Number of live females		
Generation	M.S.†	C	R	C − R	C	R	C − R	C	R	C − R
1	O	7.64	3.69	3.95±.51**	4.00	1.91	2.09±.39**	3.64	1.77	1.87±.37**
2	O	7.99	6.89	1.10±.43*	4.11	3.17	0.94±.34**	3.88	3.72	0.16±.35
3	O	8.33	7.64	0.69±.44	3.97	3.62	0.35±.33	4.36	4.02	0.34±.34
3	I	8.49	7.51	0.98±.48*	4.18	3.58	0.60±.39	4.31	3.93	0.38±.34
4	O	7.66	7.20	0.46±.51	3.75	3.69	0.06±.35	3.91	3.51	0.40±.36
4	I	8.15	6.58	1.57±.55**	3.92	3.75	0.17±.40	4.23	2.82	1.41±.37**
5	O	8.03	7.09	0.94±.36**	4.08	3.56	0.52±.28	3.95	3.53	0.42±.27
5	I	7.52	6.45	1.07±.66	3.62	3.35	0.27±.51	3.90	3.10	0.80±.53
6	O	7.62	6.75	0.87±.34**	3.88	3.34	0.54±.25*	3.74	3.41	0.33±.25
7	O	6.42	6.05	0.37±.37	3.08	2.95	0.13±.26	3.34	3.10	0.24±.25
7	I	7.20	5.83	1.37±.42**	3.54	2.79	0.75±.29*	3.66	3.04	0.62±.35
8	O	6.85	5.94	0.91±.34	3.36	3.04	0.32±.24	3.50	2.90	0.60±.24**
9	O	7.30	7.10	0.20±.28	3.67	3.67	0.00±.20	3.63	3.42	0.21±.20
10	O	6.54	5.10	1.44±.43**	3.14	2.59	0.55±.32	3.39	2.51	0.88±.30**
10	I	6.62	5.12	1.50±.45**	3.31	2.30	1.01±.30**	3.31	2.82	0.49±.34
11	O(1)‡	6.85	6.87	−.02±.42	3.39	3.52	−.13±.32	3.46	3.34	0.12±.32
11	O(2)	7.42	7.08	0.34±.56	3.43	3.87	−.44±.42	3.99	3.21	0.78±.42
12	O(1)	6.31	6.43	−.12±.44	3.00	3.33	−.33±.30	3.31	3.10	0.21±.28
12	I(1)	7.02	6.23	0.79±.50	3.58	3.07	0.51±.39	3.43	3.16	0.27±.38
12	O(2)	7.68	7.24	0.44±.72	3.91	3.81	0.10±.57	3.77	3.43	0.34±.52
12	I(2)	7.83	7.65	0.18±.49	3.66	3.95	−.29±.38	4.17	3.70	0.47±.36

† M.S. = mating system; O = "outbred"; I = "inbred".
‡ Mating group (See EXPERIMENTAL PROCEDURES).
** $P < .01$
* $P < .05$

an accurate measure of the radiation induced lethal effect when the dam is irradiated. Radiation acts as a superovulating agent for a short period of time following exposure and as a sterilizing agent over longer periods of time. Clearly, the average litter size from females that have pupped throughout the post-irradiation fertile period, as was the case in generations 1 through 8 and 10 of this study, is confounded with any somatic effects on the reproductive capabilities of the dam. For this reason, the simple differences between the control and irradiated groups in these generations may be due to the induction of lethal mutations, sterility and other somatic effects. However, in the generations in which both "outbred" and "inbred" comparisons are available, valid mutation estimates should be possible by use of the interaction between treatment group and mating system, as described earlier (equation 3).

The differences between the control and irradiated groups in the total number of live young and in the numbers of live males and females per litter, in the generations in which the irradiated line females were not irradiated (generations 9, 11 and 12), were not significant (Table 2). However, the total number of live

TABLE 3

Adjusted means of the total number of dead males and dead females per litter in the control (C) and irradiated (R) groups

Generation	M.S.†	Number of dead males			Number of dead females			Number of litters	
		C	R	C − R	C	R	C − R	C	R
1	O	0.06	0.03	0.03±.06	0.15	0.02	0.13±.08	76	37
2	O	0.16	0.08	0.08±.09	0.17	0.12	0.05±.08	83	49
3	O	0.23	0.43	−.20±.12	0.32	0.17	0.15±.10	89	70
3	I	0.17	0.23	−.06±.10	0.24	0.43	−.19±.14	81	44
4	O	0.07	0.03	0.04±.05	0.13	0.00	0.13±.05**	70	72
4	I	0.02	0.13	−.11±.11	−.01	0.09	−.10±.05*	57	47
5	O	0.17	0.08	0.09±.07	0.15	0.08	0.07±.06	86	75
5	I	0.25	0.30	−.05±.19	0.16	0.54	−.38±.20*	37	26
6	O	0.13	0.14	−.01±.06	0.08	0.11	−.03±.04	110	109
7	O	0.15	0.17	−.02±.08	0.23	0.33	−.10±.10	103	96
7	I	0.06	0.23	−.17±.07	0.08	0.22	−.14±.08	75	53
8	O	0.23	0.21	0.02±.07	0.25	0.28	−.03±.09	133	92
9	O	0.10	0.11	−.01±.05	0.09	0.07	0.02±.03	228	131
10	O	0.27	0.11	0.16±.11	0.19	0.12	0.07±.08	92	51
10	I	0.16	0.21	−.05±.10	0.22	0.21	0.01±.11	74	51
11	O(1)‡	0.24	0.17	0.07±.09	0.26	0.21	0.05±.12	77	54
11	I(2)	0.22	0.21	0.01±.17	0.11	0.11	0.00±.10	45	39
12	O(1)	0.37	0.21	0.16±.10	0.44	0.26	0.18±.15	94	73
12	I(1)	0.24	0.39	−.15±.17	0.28	0.25	0.03±.12	58	43
12	O(2)	0.01	0.13	−.12±.09	0.05	0.23	−.18±.11	28	29
12	I(2)	0.05	0.03	0.02±.03	0.03	0.08	−.05±.05	65	66

† M.S. = mating system; O = "outbred"; I = "inbred".
‡ Mating group (See EXPERIMENTAL PROCEDURES).
** $P < .01$
* $P < .05$

young was in general larger in the controls than in the irradiated groups in these three generations suggesting a mutation effect of irradiation. The C−R values of Table 2 indicate that most of the differences in these three generations in total live litter size was due to a consistently smaller number of females in the irradiated groups.

The numbers of dead males and dead females per litter are presented in Table 3. The C−R values in generations 1 through 8 and 10, like those for live litter size, represent both the somatic and genetic effects of the X-ray exposure. The data from generations 9, 11 and 12 show no evidence of an increase in the number of perinatal deaths.

Data were also collected on numbers per litter at 21 days and at 69 days of age. Size of litter following the 69th day was not considered since the irradiated line females received their first X-ray exposure on day 70. Multiple regressions of the number in the litter at 21 days and at 69 days of age on radiation group (irradiated litters coded 0, controls 1), on age of dam and on age of dam squared were used to adjust these traits for the effects of age of dam.

Estimates of the number of induced lethals affecting four traits, i.e., the total

TABLE 4

Estimates of the numbers of induced autosomal recessive lethals affecting survival, per genome per r

Generation	$m \pm s_m$† T.B.‡	L.B.§	L. 21‖	L. 69¶
3	3.5 ± 14.1	7.4 ± 15.3	—3.4 ± 18.9	5.2 ± 22.3
4	7.7 ± 11.3	13.6 ± 11.7	12.3 ± 14.2	17.0 ± 16.1
5	—2.5 ± 5.6	1.7 ± 6.6	—10.4 ± 11.0	—11.3 ± 12.5
7	3.9 ± 3.9	5.4 ± 4.1	1.9 ± 5.8	—1.5 ± 6.7
10	—1.0 ± 2.9	0.2 ± 3.2	—.5 ± 4.9	2.3 ± 5.4
12(1)††	0.8 ± 1.8	2.7 ± 2.0	5.4 ± 2.8	5.3 ± 3.4
12(2)	0.0 ± 2.2	—.1 ± 2.3	—2.3 ± 3.2	—4.7 ± 4.4
Heterogeneity χ^2 (d.f. = 6	2.99	5.26	5.28	5.50
Pooled	0.5 ± 1.2	1.6 ± 1.3	1.5 ± 1.8	1.4 ± 2.2

† Multiply all $m \pm s_m$ values by 10^{-4}.
‡ Total litter size (dead plus live) at one day of age.
§ Number alive in litter at one day of age.
‖ Number in litter at 21 days of age.
¶ Number in litter at 69 days of age.
†† Mating groups 1 and 2.

number of young born, the number of live young at one day of age, the number at 21 days and at 69 days were made for each generation (Table 4). The estimates from each trait for the different generations were tested for heterogeneity and pooled by the methods described by HAVENSTEIN *et al.* (1968).

The estimate of mutation rate (see equation 3) based on total litter size at one day of age (T.B., Table 4), i.e., dead plus live, should reflect the number of induced lethals which were brought to expression by the inbreeding during the early prenatal period. Deaths which occurred late in gestation were presumably counted among the dead young at one day of age, and would therefore not be included in the T.B. estimate of mutation rate. These deaths would, however, be reflected in the estimate from the number of live young at one day of age (L.B., Table 4), i.e., the L.B. estimate should reflect both the number of prenatal and perinatal lethals.

The estimates from the different generations on size of litter at 21 days of age (L. 21) and at 69 days of age (L. 69) were not found to be heterogeneous.

The pooled estimates (Table 4) and their 95% confidence limits are given in Table 5. Estimates from the mouse for high dose-rate spermatogonial irradiation are given for comparison. Estimates of the rate of induction of recessive lethals in mouse oocytes are not available.

The estimates thought to be most comparable on the basis of time of expression (Table 5) are those from the total litter size (T.B.) from the present experiment and those from the mouse. The present estimate is based on the litter size at birth whereas those of LÜNING (1964) and LYON, PHILLIPS and SEARLE (1964) are based on live embryo corpora lutea counts. It has been argued that the latter

TABLE 5

Estimates of the rate of induction of autosomal recessive lethals, following high dose-rate X irradiation, per genome per r

Reference	Time of expression	Estimate†	95% confidence limit†	
			Lower	Upper
	Rat oocyte			
Table 4 (T.B.)	prenatal	0.5	< 0	2.7
Table 4 (L.B.)	pre- and perinatal	1.6	< 0	4.1
Table 4 (L. 21)	pre- peri- and postnatal	1.5	< 0	5.0
Table 4 (L. 69)	pre- peri- and postnatal	1.4	< 0	5.7
	Mouse spermatogonia			
LÜNING (1964)	prenatal	0.8–2.0	< 0	6.8
LYON *et al.* (1964)	prenatal	2.5	0.6	4.6

† Multiply each value by 10^{-4}.

method is more accurate (RUSSELL and RUSSELL 1954; LÜNING 1964). The estimate made by LÜNING and the present rat estimate are based on data from sib and non-sib crosses, whereas the one made by LYON *et al.* (1964) is based on embryo survival in F_2 females mated to their sires. Nevertheless, the estimates obtained from mouse spermatogonia and rat oocytes by these different methods are similar.

It should be noted that RUSSELL (1965) has recently shown that the interval of time from irradiation to conception has a large effect on the frequency of induced mutations observed. In the present experiment the females were not mated until all had received their third X-ray fraction. This resulted in an average irradiation-to-conception interval for the different generations of 7 to 11 weeks following the first exposure (10 weeks of age) and 3 to 7 weeks following the third exposure (14 weeks of age). Thus, if the irradiation-to-conception interval effect is similar in the rat and the mouse, the present results would be underestimates of the mutation rates expected if mated shortly after irradiation. They presumably represent an average from the entire post-irradiation fertile period.

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SUMMARY

Estimates of the numbers of induced autosomal recessive lethals affecting the size of the litter at birth and postnatal survival were made following nine generations of cumulative maternal pre-fertilization X irradiation. The data provide an upper 95 percent confidence limit of 4.1×10^{-4} induced lethals per genome per roentgen of exposure for pre- and perinatal lethals affecting the live litter size at one day of age. Data on litter size at 21 days of age and at 69 days of age

provided upper 95 percent confidence limits of 5.0×10^{-4} and 5.7×10^{-4} induced lethals per genome per r, respectively. The relationship of these estimates to those from the mouse is discussed.

LITERATURE CITED

CHAPMAN, A. B., J. L. HANSEN, G. B. HAVENSTEIN, and N. E. MORTON, 1964 Genetic effects of cumulative irradiation on prenatal and early postnatal survival in the rat. Genetics **50:** 1029–1042.

CHUNG, C. S., and A. B. CHAPMAN, 1958 Comparisons of the predicted with actual gains from selection of parents of inbred progeny of rats. Genetics **43:** 594–600.

FISHER, R. A., 1966 *Design of Experiments.* 8th ed., Hafner, N.Y.

HAVENSTEIN, G. B., B. A. TAYLOR, J. L. HANSEN, N. E. MORTON, and A. B. CHAPMAN, 1968 Genetic effects of cumulative X-irradiation on the secondary sex ratio of the laboratory rat. Genetics **59:** 255–274.

KYLE, W. H., and A. B. CHAPMAN, 1953 Experimental check of the effectiveness of selection for a quantitative character. Genetics **38:** 421–443.

LÜNING, K. G., 1964 Studies of irradiated mouse populations. III. Accumulation of recessive lethals. Mutation Res. **1:** 86–98.

LYON, M. F., R. J. S. PHILLIPS, and A. B. SEARLE, 1964 The overall rates of dominant and recessive lethal and visible mutations induced by spermatogonial X-irradiation of mice. Genet. Res. **5:** 448–467.

RUSSELL, L. B., and W. L. RUSSELL, 1954 Pathways of radiation effects in the mother and embryo. Cold Spring Harbor Symp. Quant. Biol. **19:** 50–59.

RUSSELL, W. L., 1965 Effect of the interval between irradiation and conception on mutation frequency in female mice. Proc. Natl. Acad. Sci. U.S. **54:** 1552–1557.

SNEDECOR, G. W., 1956 *Statistical Methods.* 5th ed., Iowa State College Press, Ames.