GENETIC EFFECTS OF SPERMATOGONIAL IRRADIATION ON GROWTH AND AGE AT SEXUAL MATURITY IN RATS

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INTEREST in the genetic effects of irradiation on quantitative characters usually takes the form of two questions. Does irradiation effect a genetic change in the mean of quantitative characters such as human intelligence? Does irradiation induce genetic variance in quantitative characters that could be utilized by the plant or animal breeder for genetic improvement?

Experimental data relevant to these questions are scanty, especially in mammals, and the results of different experimenters are frequently inconsistent.

The present data were collected in a long-term experiment designed primarily to estimate the frequency of induction of mutations affecting fitness.

MATERIALS AND METHODS

Most of the details of the experimental procedures have been given previously (TAYLOR and CHAPMAN 1969). Briefly, the experiment consisted of comparing an irradiated population with a contemporaneous control population. Both populations originated in the same highly inbred line of rats (MI₄). The males of the treated line received a fractionated 450r dose (*d*) of X rays each generation administered as 100, 150, and 200r fractions at 10, 12, and 14 weeks of age. These lines were continued by restricted random mating. Additional matings were made between full sibs in generations 3, 4, and 6 to 14.

Body weights were taken at 21, 42, and 69 days of age. Body weights were recorded to the nearest gram. Beginning at 28 days of age, females were checked daily for the opening of the vagina. This was considered to be an indication of sexual maturity.

THEORY AND METHODS OF ANALYSIS

Assumptions: No clear pattern for the effect of irradiation on quantitative characters in mammals has as yet been established. As a working hypothesis the following model will be assumed:

(i) Mutations affecting body weight and age at vaginal opening are neutral with respect to fitness. (ii) These mutations do not exhibit epistasis. (iii) Genotype-environment interaction is unimportant within the range of environments encountered in this experiment. (iv) The effects of sex-linked mutations are negligible.

Accumulation of mutations affecting quantitative characters: Since only one parent is irradiated, the average amount of cumulative radiation exposure in the

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treated line in the n th generation is nd in the absence of selection, where Generation 1 denotes the first generation whose sires were treated and d is the dose of radiation administered to males each generation in roentgens.

In generation n of the irradiated inbred group a fraction F of the mutations inherited by generation $n - 2$ would be expected to have become homozygous where F is the inbreeding coefficient. The remaining fraction $(1 - F)$ of the mutations would be in the heterozygous condition in generation n as would the mutations that occurred in the two preceding generations. The amount of irradiation exposure relevant to heterozygous effects in the irradiated groups in generation n is:

$$\begin{aligned} A_n &= (1-F) (n-2)d + 2d \\ &= [n-F(n-2)]d. \end{aligned}$$

By similar reasoning the amount of radiation exposure put to test in the irradiated inbreds of generation n relevant to the homozygous effects of mutations is:

$$a_n = F(n-2)d/2.$$

Spontaneous mutations exhibiting directional dominance would tend to differentiate the inbreds from the outbreds. HALDANE (1936) has calculated that in the absence of selection, the probability of heterozygosity at a locus in a line maintained by full-sib mating for many generations is $12m'_i$, where m'_i is the mutation rate. After the beginning of the experiment (and restricted random mating), the amount of heterozygosity would increase by $2m'_i$ each generation. If we assume that dominance is complete and unidirectional, the exposure to recessive mutations affecting quantitative characters would be approximately:

$$a'_n = F(n+8).$$

The effects of spontaneous and induced mutations on the mean of the character can be expressed in the following terms:

Let m_i be the induced mutation rate per roentgen at the i th locus to an allele with homozygous effect t_i and heterozygous effect $h_i t_i$ on the character, and let m'_i be the spontaneous mutation rate at the i th locus to a recessive allele with homozygous effect t'_i . Summing over all loci, the expected deviation of the mean of a particular group from the mean of the control outbreds in the n th generation is:

$$\Delta_n = \sum m_i h_i t_i A_n + \sum m_i t_i a_n + \sum m'_i t'_i a'_n.$$

The quantities $\sum m_i h_i t_i$, $\sum m_i t_i$, and $\sum m'_i t'_i$ can be estimated from the data, and the ratio

$$\bar{h} = \frac{\sum m_i h_i t_i}{\sum m_i t_i}$$

may be regarded as an estimate of the average dominance. This average is weighted by $m_i t_i$ and is meaningful only if the t_i 's are essentially all of the same sign. If the t_i 's are negative, $\bar{h}$'s of one, zero, or less than zero would indicate complete dominance, complete recessivity, or overdominance of induced mutations, respectively. On the other hand, if the t_i 's are positive, $\bar{h}$'s of zero, one, or greater

than one would imply complete recessivity, complete dominance, or overdominance, respectively.

Statistical methods: The traits under study, body weights and age at vaginal opening, may be affected by many environmental as well as genetic circumstances. For this reason, comparisons are made within generation-mating group, and the sexes are analyzed separately. Littermates share a common environment, so variation between litters is taken into account in analysis of between-group differences. The traits are adjusted for parity, age of dam, litter size at birth, and litter size at 21 days by multiple covariance analysis.

The effects of mutations on the means are estimated by making orthogonal comparisons within each generation-mating group, and regressing the differences

on $a_n = \frac{F(n-2)d}{2}$, $A_n = [n-F(n-2)]d$ and $a'_n = F(n+8)$ in a weighted multiple regression analysis through the origin. The test for nonlinearity should reflect whether or not natural selection, epistasis, genotype-environment interaction, and/or other complicating factors are playing roles in the changes from generation to generation. This method of analysis is essentially the same as the method used previously for litter-size data (TAYLOR and CHAPMAN 1969).

Two methods are used to test for an increase in variance due to irradiation. The first method compares the within-litter variance of control and irradiated groups. The comparisons are made within mating systems for each different mating group. Any increase observed in the outbred comparison would reflect one half of the variation due to heterozygous effects. The inbred comparisons should reflect portions of both heterozygous and homozygous effects of induced mutations on variance.

The other method of detecting a variance increase involves calculating the regression of offspring on parent for the different traits. This method permits the estimation of the additive variance increase.

Under random mating the regression of offspring on sire is:

$$b_{os} = \frac{0.5 \sigma_A^2}{\sigma_T^2}$$

The corresponding offspring-on-dam regression is:

$$b_{od} = \frac{0.5 \sigma_A^2 + \sigma_{ME}}{\sigma_T^2}$$

where: σ_A^2 is the additive genetic variance, σ_{ME} is the covariance between the environmental deviation of the dam and the environmental deviation of the offspring, and σ_T^2 is the total phenotypic variance. Covariances between parent and offspring due to different levels of additive $\times$ additive epistatic effects, due to transmitted and direct maternal effects, and to variance from direct maternal effects are assumed to be unimportant contributors to these regressions. The regression coefficients were calculated as the ratio of the covariance between off-

TABLE 1

Analysis of variance and covariance illustrating the procedure used in estimating the regression of offspring on parent

Source	df	Sums of squares		Cross products
		Parent	Offspring	
Between parents	$p-1$	Σx^2_B	Σy^2_B	Σxy_B
Regression of offspring on parent	1		$b^2_{op} \Sigma x^2_B$	
Deviations from regression	$p-2$		$\Sigma y^2_B - b^2_{op} \Sigma x^2_B$	
Within parents	$N-p$		Σy^2_W	
$b_{op} = \frac{\Sigma xy_B}{\Sigma x^2_B}$			$s_b^2 = \frac{(\Sigma y^2_B - b^2_{op} \Sigma x^2_B) / (p-2)}{\Sigma x^2_B}$	

spring and parental records, where each offspring record was given equal weight and each parental record was repeated for each offspring in calculating the sums of squares and cross products. Table 1 shows the analysis of variance and covariance procedure. N is the total number of animals, p is the number of parental families, Σx^2_B , Σy^2_B and Σxy_B are the corrected sums of squares and cross products among parental families. Σy^2_W is the sum of squares among progeny within the same parental family.

Individuals which lacked a record (mortality, missing on weigh day, etc.) for any of the traits, were excluded from the analysis. This tended to exclude unhealthy animals and also facilitated the analysis. Selection of the data in this way should make these results largely independent of effects on survival which are analyzed by different methods.

RESULTS AND DISCUSSION

Adjustment of the data: The analysis of covariance provides a test for the homogeneity of regression coefficients among the different classes. The test was carried out for each of the traits in each mating group. The assumption of homogeneity was not disputed statistically in 72 of 75 cases in the male analyses at the 95% confidence level. For the female data, the assumption of homogeneity was not disproved in 89 of 99 tests. Although the latter frequency of significance was about twice that expected by chance, "significance" is questionable since not all of these tests were independent. It is concluded that some heterogeneity may exist but it seems doubtful if it is large enough to invalidate the subsequent analyses. The means of the different classes were therefore adjusted, and the variances of the adjusted means were calculated.

Effects on means: The adjusted means are presented in Tables 2 through 4. Differences between control and irradiated means within mating group and mating system are also presented. The signs of these differences for the different traits show a similar pattern in both sexes; the irradiated animals in most cases

TABLE 2
Average adjusted male body weights at 21, 42 and 69 days of age, within generations, mating group, and mating system, in the control (C) and irradiated (R) groups*

Generation	Mating group	Mating system†	Number of rats			Mean 21-day weight (g)			Mean 42-day weight (g)			Mean 69-day weight (g)		
			C	R		C	R	C-R	C	R	C-R	C	R	C-R
1	1	O	177	222		36.0	35.3	0.7 ± 1.0	121.9	122.1	-0.2 ± 3.1	225.8	224.7	1.1 ± 4.7
1	2	O	66	71		38.3	36.8	1.5 ± 2.1	135.0	131.5	3.4 ± 5.9	249.9	247.8	2.2 ± 7.7
2	1	O	354	341		31.5	32.3	-0.8 ± 0.5	116.4	120.2	-3.8 ± 1.8	213.6	218.0	-4.4 ± 2.2
2	2	O	85	161		37.9	37.0	1.0 ± 1.8	135.1	132.6	2.5 ± 4.5	236.7	233.9	2.8 ± 6.6
3	1	O	265	351		32.8	33.8	-1.0 ± 0.6	125.2	127.0	-1.7 ± 1.8	221.3	222.9	-1.6 ± 2.5
3	1	I	118	210		32.9	33.4	-0.5 ± 0.9	126.3	127.0	-0.8 ± 2.5	220.8	221.6	-0.8 ± 3.5
3	2	O	54	47		31.3	34.4	-3.1 ± 2.9	123.4	124.5	-1.1 ± 9.2	245.4	230.1	15.3 ± 13.4
4	1	O	164	106		32.8	32.3	0.5 ± 1.1	115.8	116.5	-0.8 ± 2.7	219.8	218.9	0.9 ± 4.5
4	1	I	62	31		34.1	33.3	0.8 ± 1.7	116.7	119.1	-2.3 ± 4.8	221.9	217.3	4.6 ± 7.9
5	1	O	268	179		28.1	29.0	-0.9 ± 0.7	101.7	105.7	-4.0 ± 2.2	190.1	195.4	-5.3 ± 3.5
5	2	O	211	143		32.7	34.1	-1.3 ± 0.8	114.0	118.4	-4.4 ± 2.2	210.2	218.2	-8.0 ± 3.3
6	1	O	253	235		30.8	30.8	-0.0 ± 0.6	114.0	112.6	1.4 ± 1.9	213.3	210.2	3.0 ± 2.9
6	1	I	110	97		30.3	30.3	0.0 ± 0.9	115.4	109.7	5.6 ± 2.9	213.5	203.8	9.7 ± 4.4
6	2	O	223	255		34.6	33.4	1.2 ± 0.6	125.1	120.9	4.2 ± 1.7	214.6	208.8	5.9 ± 2.8
7	1	O	285	186		31.1	31.8	-0.8 ± 0.7	110.2	108.9	1.3 ± 2.3	205.5	209.1	-3.6 ± 3.7
7	1	I	46	30		32.0	32.6	-0.6 ± 1.7	112.6	114.6	-2.0 ± 5.7	210.5	211.8	-1.2 ± 9.2
7	2	O	166	101		31.3	30.9	0.4 ± 0.9	109.1	111.0	-2.0 ± 3.4	199.9	205.0	-5.1 ± 5.6
7	2	I	49	36		33.7	29.5	4.2 ± 1.6	113.3	112.1	1.1 ± 5.9	216.4	214.9	1.5 ± 9.7
8	1	O	230	205		32.3	33.5	-1.3 ± 0.7	112.6	116.1	-3.4 ± 2.2	199.7	203.4	-3.6 ± 4.0

TABLE 2—Continued

Generation	Mating group	Mating system†	Number of rats		Mean 21-day weight (g)			Mean 42-day weight (g)			Mean 69-day weight (g)		
					C	R	C-R	C	R	C-R	C	R	C-R
			C	R	C	R	C-R	C	R	C-R	C	R	C-R
8	1	I	79	86	32.9	33.3	-0.4 ± 1.1	116.1	114.8	1.3 ± 3.5	208.4	201.1	7.3 ± 6.5
8	2	O	161	145	30.6	31.8	-1.1 ± 1.0	103.8	107.6	-3.8 ± 3.3	202.8	205.0	-2.2 ± 5.4
9	1	O	286	176	31.1	33.1	-2.0 ± 0.8	108.5	112.8	-4.3 ± 2.5	206.5	212.9	-6.4 ± 3.6
9	1	I	85	25	32.0	31.5	0.5 ± 1.9	114.0	117.2	-3.2 ± 6.0	211.3	215.3	-4.0 ± 8.4
9	2	O	314	251	33.2	34.7	-1.5 ± 0.7	114.4	118.8	-4.5 ± 2.0	214.0	212.8	1.2 ± 3.1
10	1	O	361	232	30.3	31.7	-1.5 ± 0.7	106.2	110.2	-4.0 ± 2.5	190.9	194.1	-3.3 ± 4.1
10	1	I	104	94	31.3	34.6	-3.3 ± 1.2	109.2	118.3	-9.1 ± 4.2	193.9	206.2	-12.3 ± 7.0
11	1	O	146	148	29.4	30.0	-0.5 ± 0.9	102.6	106.0	-3.4 ± 2.8	199.7	203.6	-3.9 ± 4.6
11	1	I	176	146	29.4	29.2	0.2 ± 0.8	105.0	104.2	0.8 ± 2.7	203.5	199.6	4.0 ± 4.4
11	2	O	68	58	32.5	35.3	-2.8 ± 1.5	108.1	115.8	-7.7 ± 4.1	202.6	202.0	0.7 ± 7.3
12	1	O	151	153	29.0	30.0	-1.0 ± 0.8	103.3	106.6	-3.3 ± 2.5	190.4	194.5	-4.1 ± 4.8
12	1	I	139	123	29.9	31.7	-1.8 ± 0.9	106.7	110.2	-3.5 ± 2.7	192.2	199.0	-6.8 ± 5.2
12	2	O	68	35	32.0	34.5	-2.6 ± 1.5	101.6	115.1	-13.5 ± 6.0	198.6	223.2	-24.6 ± 10.0
12	2	I	80	87	31.3	34.3	-3.0 ± 1.1	105.7	107.1	-1.4 ± 4.4	204.1	206.8	-2.7 ± 7.4
13	1	O	95	103	32.1	33.5	-1.4 ± 1.0	108.8	113.1	-4.3 ± 3.6	204.0	211.5	-7.6 ± 5.4
13	1	I	59	39	33.6	33.6	-0.0 ± 1.5	111.0	114.1	-3.1 ± 5.2	201.8	211.7	-9.8 ± 7.8
13	2	O	49	40	35.3	38.3	-3.1 ± 2.1	116.0	122.9	-7.0 ± 6.6	210.4	218.1	-7.7 ± 9.2
13	2	I	69	45	37.3	35.8	1.5 ± 1.9	121.8	122.0	-0.3 ± 5.9	215.4	214.1	1.3 ± 8.3
14	1	I	99	80	31.7	33.1	-1.4 ± 1.0	105.3	107.6	-2.2 ± 3.9	204.1	208.9	-4.9 ± 5.9

* Adjusted for age of dam, age of dam squared, parity, litter size at birth, and litter size at weaning.

† O = "outbred," I = "inbred."

TABLE 3

*Average adjusted female body weights at 21 and 42 days of age, within generation, mating group, and mating system, in the control (C) and irradiated (R) groups**

Gener- ation	Mating group	Mating system†	Number of rats		Mean 21-day weight (g)			Mean 42-day weight (g)		
			C	R	C	R	C-R	C	R	C-R
1	1	O	180	182	35.3	33.9	1.4 ± 1.1	102.9	102.8	0.1 ± 2.1
1	2	O	52	82	36.0	37.0	-1.0 ± 2.2	106.1	107.3	-1.2 ± 4.6
2	1	O	333	386	30.5	31.0	-0.5 ± 0.5	97.9	98.7	-0.8 ± 1.3
2	2	O	74	166	36.9	35.4	1.5 ± 1.4	107.5	107.5	0.0 ± 2.4
3	1	O	323	386	32.4	32.9	-0.5 ± 0.5	103.2	103.2	-0.1 ± 1.0
3	1	I	128	230	32.3	32.6	-0.3 ± 0.8	102.5	103.5	-1.0 ± 1.5
3	2	O	71	60	30.4	35.3	-4.8 ± 1.9	99.9	107.9	-8.0 ± 3.5
4	1	O	130	126	31.7	32.1	-0.3 ± 1.0	95.5	96.6	-1.1 ± 2.0
4	1	I	72	37	31.8	32.8	-1.1 ± 1.7	99.3	101.4	-2.0 ± 3.2
5	1	O	292	197	27.1	28.7	-1.6 ± 0.7	85.7	88.9	-3.3 ± 1.6
5	2	O	225	148	32.3	32.3	-0.0 ± 0.7	94.8	95.6	-0.8 ± 1.5
6	1	O	252	229	29.5	29.8	-0.2 ± 0.6	94.2	93.7	0.5 ± 1.2
6	1	I	125	96	29.5	28.5	1.1 ± 0.8	94.2	93.0	1.2 ± 1.8
6	2	O	219	271	32.3	31.8	0.5 ± 0.7	98.8	98.2	0.6 ± 1.5
7	1	O	315	227	30.5	30.5	0.1 ± 0.6	93.9	92.7	1.2 ± 1.3
7	1	I	34	41	30.5	29.8	0.7 ± 1.6	93.3	98.4	-5.2 ± 3.4
7	2	O	160	103	30.9	30.0	1.0 ± 0.9	93.3	95.5	-2.3 ± 2.3
7	2	I	43	39	30.2	28.7	1.5 ± 1.5	91.2	89.3	2.0 ± 4.0
8	1	O	261	227	31.0	32.0	-1.0 ± 0.6	93.4	97.2	-3.8 ± 1.6
8	1	I	86	68	32.3	31.2	1.1 ± 1.1	97.5	96.3	1.2 ± 2.8
8	2	O	160	162	30.4	29.9	0.6 ± 0.9	91.9	91.5	0.4 ± 2.2
9	1	O	268	202	29.7	31.4	-1.6 ± 0.7	92.6	96.0	-3.4 ± 1.5
9	1	I	102	37	29.6	29.8	-0.2 ± 1.5	92.8	92.1	0.7 ± 3.1
9	2	O	347	266	31.8	32.5	-0.6 ± 0.6	96.7	97.7	-1.1 ± 1.4
10	1	O	368	245	29.3	30.4	-1.1 ± 0.7	90.7	94.6	-3.9 ± 1.7
10	1	I	119	104	30.6	32.9	-2.3 ± 1.1	92.7	98.8	-6.1 ± 2.8
11	1	O	150	171	28.8	28.8	0.0 ± 0.8	90.7	91.8	-1.1 ± 1.9
11	1	I	177	142	28.0	28.4	-0.3 ± 0.8	89.8	90.3	-0.4 ± 1.9
11	2	O	68	57	31.4	31.9	-0.5 ± 1.6	89.5	91.4	-1.9 ± 2.8
12	1	O	187	169	28.8	28.5	0.3 ± 0.7	90.1	90.3	-0.2 ± 1.8
12	1	I	127	119	29.6	30.8	-1.2 ± 0.9	88.9	93.4	-4.6 ± 2.1
12	2	O	55	41	32.2	33.6	-1.4 ± 1.7	92.8	98.7	-5.9 ± 5.0
12	2	I	86	97	30.2	31.5	-1.3 ± 1.2	89.6	88.9	0.7 ± 3.6
13	1	O	126	111	32.1	31.7	0.4 ± 0.9	93.7	95.5	-1.7 ± 2.2
13	1	I	64	58	31.6	33.1	-1.4 ± 1.3	92.9	96.6	-3.7 ± 3.1
13	2	O	36	43	34.8	34.2	0.6 ± 2.0	103.6	101.2	2.4 ± 4.4
13	2	I	61	52	37.3	34.8	2.5 ± 1.6	101.8	97.8	3.9 ± 3.7
14	1	I	107	86	31.8	32.6	-0.9 ± 1.0	91.5	93.5	-2.0 ± 3.1

* Adjusted for age of dam, age of dam squared, parity, litter size at birth, and litter size at weaning.

† O = "outbred," I = "inbred."

are heavier, and the age at vaginal opening was less in irradiated females. In many cases the differences are significant.

The adjusted means were analyzed to estimate the average heterozygous and

TABLE 4

*Average adjusted 69-day body weights and age at vaginal opening for females of control (C) and irradiated (R) groups within generation, mating group, and mating system**

Gener- ation	Mating group	Mating system†	Number of rats		Mean 69-day weight (g)			Mean age at vaginal opening(days)		
			C	R	C	R	C-R	C	R	C-R
1	1	O	180	182	161.6	160.9	0.7 ± 2.4	35.7	35.8	-0.2 ± 0.4
1	2	O	52	82	168.6	169.5	-0.9 ± 4.8	34.7	35.7	-1.0 ± 0.7
2	1	O	333	386	155.9	157.0	-1.1 ± 1.4	37.4	37.3	0.2 ± 0.4
2	2	O	74	166	163.9	164.4	-0.5 ± 3.1	35.8	36.1	-0.3 ± 0.5
3	1	O	323	386	157.2	157.3	-0.1 ± 1.2	35.7	35.6	0.1 ± 0.2
3	1	I	128	230	156.4	158.0	-1.5 ± 1.8	35.7	35.4	0.2 ± 0.3
3	2	O	71	60	157.6	166.5	-8.9 ± 5.1	35.3	35.0	0.3 ± 0.6
4	1	O	130	126	151.8	151.9	-0.1 ± 2.1	37.6	37.9	-0.3 ± 0.6
4	1	I	72	37	156.4	158.8	-2.4 ± 3.4	37.6	36.9	0.7 ± 1.0
5	1	O	292	197	138.9	143.2	-4.3 ± 1.8	40.5	40.0	0.5 ± 0.5
5	2	O	225	148	150.2	151.3	-1.0 ± 1.6	37.8	37.3	0.5 ± 0.4
6	1	O	252	229	148.1	148.7	-0.6 ± 1.4	38.0	38.6	-0.6 ± 0.3
6	1	I	125	96	148.8	147.2	1.7 ± 2.1	37.8	38.7	-0.9 ± 0.5
6	2	O	219	271	148.0	148.5	-0.5 ± 1.8	38.1	38.0	0.1 ± 0.4
7	1	O	315	227	150.7	147.3	3.4 ± 1.9	37.8	39.0	-1.2 ± 0.4
7	1	I	34	41	150.9	156.8	-5.9 ± 5.0	37.4	37.0	0.4 ± 0.9
7	2	O	160	103	149.3	150.3	-1.0 ± 2.8	38.9	38.6	0.2 ± 0.7
7	2	I	43	39	148.5	139.4	9.1 ± 4.9	38.6	40.1	-1.5 ± 1.2
8	1	O	261	227	142.4	148.0	-5.6 ± 1.9	38.3	36.9	1.4 ± 0.4
8	1	I	86	68	148.2	142.8	5.4 ± 3.4	37.0	36.1	0.9 ± 0.8
8	2	O	160	162	148.0	148.3	-0.4 ± 2.8	37.9	37.6	0.2 ± 0.6
9	1	O	268	202	145.4	152.1	-5.7 ± 1.8	37.7	36.7	1.0 ± 0.4
9	1	I	102	37	145.1	143.9	2.3 ± 3.6	37.3	37.3	-0.0 ± 0.8
9	2	O	347	266	151.1	152.0	-0.9 ± 1.8	36.6	36.1	0.4 ± 0.4
10	1	O	368	245	139.1	143.2	-4.2 ± 2.2	38.4	38.0	0.4 ± 0.5
10	1	I	119	104	138.5	147.9	-9.5 ± 3.5	38.7	37.5	1.1 ± 0.8
11	1	O	150	171	144.1	146.3	-2.2 ± 2.5	38.2	37.8	0.3 ± 0.6
11	1	I	177	142	143.2	142.6	0.6 ± 2.5	38.5	38.6	-0.0 ± 0.6
11	2	O	68	57	146.6	149.4	-2.8 ± 3.5	37.9	36.4	1.6 ± 0.9
12	1	O	187	169	143.9	143.8	0.1 ± 2.5	36.9	37.2	-0.3 ± 0.6
12	1	I	127	119	140.9	145.5	-4.7 ± 3.0	37.0	36.1	0.9 ± 0.7
12	2	O	55	41	149.4	151.5	-2.1 ± 5.5	37.4	37.5	-0.0 ± 1.4
12	2	I	86	97	142.6	146.1	-3.5 ± 3.9	39.0	39.0	-0.1 ± 1.0
13	1	O	126	111	149.2	152.6	-3.4 ± 2.8	36.8	36.8	0.0 ± 0.6
13	1	I	64	58	149.3	150.7	-1.4 ± 3.9	37.0	36.1	0.8 ± 0.9
13	2	O	36	43	155.5	158.8	-3.3 ± 5.2	36.6	36.9	-0.3 ± 1.3
13	2	I	61	52	158.1	155.9	2.1 ± 4.3	37.3	38.2	-1.0 ± 1.1
14	1	I	107	86	151.4	154.2	-2.8 ± 3.0	...	...	...

* Adjusted for age of dam, age of dam squared, parity, litter size at birth, and litter size at weaning.

† O = "outbred," I = "inbred."

homozygous effects per roentgen of cumulative exposure, as well as the average homozygous effects of spontaneous mutations. The results of these analyses are presented in Tables 5 and 6, for males and females, respectively.

TABLE 5

Average effects of induced and spontaneous mutations on male body weights

Trait	Average cumulative effect (g)			Test for nonlinearity†
	Spontaneous	Induced (/gamete/100r)		
	Homozygous effect	Heterozygous effect	Homozygous effect	
Body weight				
21-day	0.10 ± 0.07	**0.03 ± 0.01	0.02 ± 0.10	P~0.10
42-day	**0.58 ± 0.21	**0.09 ± 0.02	—0.24 ± 0.30	P~0.70
69-day	0.37 ± 0.37	0.07 ± 0.04	—0.12 ± 0.54	P~0.20

† Test of goodness of fit to the linear model.

** P < .01.

The tests for nonlinearity of regression were close to significance for the traits 21-day and 69-day weights in females. This suggests that the simple model assumed here may not be completely adequate to fit the data. Since this is a test of the entire model, it is difficult to say what, if any, particular assumption(s) is incorrect. The linear effects accounted for a significant portion of the variability in most of these analyses. It is felt that the parameters estimated in these analyses give a fairly good indication of the effects of irradiation on these traits.

The most striking result is the evidence for heterozygous effect of induced mutations. These results point to an increase in body weight in both sexes and to a decrease in age at vaginal opening.

In contrast to the heterozygous effects, the homozygous effects of induced mutations seemed to be to decrease body weights and to increase age at vaginal opening. The magnitudes of the homozygous effects are considerably greater than the heterozygous effects in most cases.

The estimated homozygous effects of spontaneous mutations was in the direc-

TABLE 6

Average effects of induced and spontaneous mutations on female body weights and age at vaginal opening

Trait	Average cumulative effect (g, day)			Test for nonlinearity†
	Spontaneous	Induced (/gamete/100r)		
	Homozygous effect	Heterozygous effect	Homozygous effect	
Body weight				
21-day	0.01 ± 0.07	0.01 ± 0.01	0.07 ± 0.11	P=0.05
42-day	0.01 ± 0.15	*0.04 ± 0.02	0.09 ± 0.23	P~0.40
69-day	0.04 ± 0.20	**0.07 ± 0.02	—0.31 ± 0.31	P~0.06
Age at vaginal opening	—0.04 ± 0.04	—0.01 ± 0.01	0.02 ± 0.07	P~0.45

† Test of goodness of fit to the linear model.

** P < .01.

tion of increased body weight and decreased age at vaginal opening. These effects attained significance only in the case of 42-day body weight of males.

Increased body weight in the progeny of irradiated males has been reported in both mice (TOUCHBERRY and VERLEY 1964) and rats (NEWCOMBE and MCGREGOR 1965). Although the direction of the effects of irradiation on body weight found in their experiments is consistent with the present results, the magnitude of the effects was greater in their studies. In their studies irradiated spermatozoa were sampled instead of spermatogonia. Since mature sperm are known to be more mutable than spermatogonia, perhaps this difference should not be considered surprising.

SCHLAGER, RODERICK and STORER (1966) studied the effect of spermatogonial irradiation on body weight in mouse populations formed by crossing four inbred lines. A single dose of 900r was given to males each generation in a series of small sub-lines. Preliminary results indicated that ancestral spermatogonial irradiation decreased body weight. Since there was no deliberate inbreeding, these effects were presumably due to the heterozygous effects of induced mutations. Their results appear to be contradictory to the present findings. GREEN (1968) studied these same lines with respect to 60-day body weight and embryonic mortality. After varying numbers of generations of irradiation, the sub-lines were maintained for several generations by random mating without additional irradiation. The "test" animals were produced by full-sib mating. A significant decrease in male body weight relative to the controls was observed. A smaller, nonsignificant decrease was observed in female body weight. Unfortunately, the experimental design did not permit the distinction between heterozygous and homozygous effects. Because of this limitation and also because the author made different assumptions about the rate of accumulation of mutants affecting body weight than are made in the present study, it is difficult to make comparisons. However, to a first approximation, his results appear to be consistent with the homozygous effects of induced mutations on 69-day weights in the present study.

Cox (1968) reported that the mean 154-day body weight of pigs whose sires were given 300r spermatogonial irradiation was 1% less than controls in the Duroc breed and 0.5% less in the Hampshire breed. Only the difference in the Duroc breed was significant. These differences are opposite to the expectation based on the increases observed in the present study.

It appears that the average heterozygous and homozygous effects of induced mutations in the present study are opposite in direction, although the significance of this is subject to question. If the effect were real, the most obvious explanation would be that induced mutations exhibit overdominance with respect to these traits. If overdominance is the principal kind of gene action, selection for increased body weight (or decreased age at vaginal opening) should lead to an intermediate equilibrium gene frequency such that when selection ceased to be effective the population would still be segregating. Such a population would be responsive to reverse selection. An alternative hypothesis would postulate two classes of induced mutations. The first class would have positive additive effects on body weight. The other class would have negative recessive effects on body

weight. If the alternative hypothesis is correct, selection for increased body weight should fix the first class of mutations and eliminate the second class leading to fixation at the selection limit. In this case reverse selection would be ineffective. Thus, selection would appear to be an effective way to distinguish between these two hypotheses as well as to answer the question as to how much selection response can be obtained after a given amount of irradiation. The latter will depend on the number of induced mutations and the magnitude of their individual effects.

Effects on within-litter variances: The within-litter mean squares for the body weights and age at vaginal opening were computed for each group classified by sex, generation-mating group, mating system and treatment. Each mean square in the irradiated groups was compared with the mean square in the corresponding control group on the basis of direction and significance of differences. The F test was used in judging significance. Comparing the frequencies of positive differences and "significant" differences from all the analyses should provide a robust test for an increase in variance. Out of the 264 comparisons, the irradiated mean square exceeded the control value 145 times (55%). If all these comparisons were independent, the probability of obtaining this many or more positive differences ($R-C$) would be about 0.05. Since not all of these comparisons are independent, the true probability is somewhat greater. The irradiated mean square was "significantly" greater ($P < .05$) than the control mean square in 32 cases, while the reverse was true in 19 cases. The chance probability of obtaining a difference as large as or larger than this and in the same direction is about 0.03. Again, it must be recalled that the assumption of independence is not strictly valid. It seems safe to conclude that an increase in variance due to irradiation is suggested by the data. The increased variances appear to be concentrated among inbred males. One would expect the variance increase to be expressed mainly in the inbreds if induced mutations are recessive, and recessive X-linked mutations would be expressed more often in males than in females.

In order to estimate any changes in variance, a weighted multiple regression analysis was performed paralleling the analysis done on the means. Differences between control and irradiated mean squares, within generation-mating groups, were regressed on the heterozygous and homozygous exposures. The results of these analyses are presented in Tables 7 and 8 for males and females, respectively. The interpretation of these analyses is complicated by the fact that since the dependent variables are variances, the assumption of normality is invalid. Therefore, the significance tests must be regarded as only approximate. Variance increases in the male body weights associated with heterozygous and homozygous radiation exposure were observed but were significant only for homozygous exposure in 21-day weights. There is little or no evidence of a variance increase in the female analysis.

Effects on offspring-parent regression: The regressions of offspring on parent for body weight and age at vaginal opening were calculated on the unadjusted data for each sub-group classified by generation, treatment line, mating system, sex of offspring and sex of parent. The regression of offspring on sire was gener-

TABLE 7

Effects of induced and spontaneous mutations on the within-litter variance of male body weights

Trait	Average cumulative effect			Test for nonlinearity†
	Spontaneous	Induced (/gamete/100r)		
	Homozygous effect	Heterozygous effect	Homozygous effect	
Body weight				
21-day	—0.18 ± 0.15	0.02 ± 0.02	*0.52 ± 0.20	P~0.30
42-day	*—10.42 ± 4.01	0.34 ± 0.45	8.74 ± 6.16	P<0.001
69-day	—20.61 ± 13.19	1.13 ± 1.41	1.17 ± 20.42	P<0.01

† Test of goodness of fit to the linear model.

* P < .05.

ally small and nonsignificant as would be expected in a highly inbred line. The regression of offspring on dam was generally larger and sometimes significant. The latter probably reflects a positive environmental covariance. This could be explained if the larger dams produced more milk for their progeny than the smaller dams.

In order to test the null hypothesis that irradiation has not increased the genetic variance, the differences between the irradiated and control regression coefficients were regressed on the cumulative irradiation exposure by a weighted regression analysis. For the outbreds, this would measure one half the increase in heritability per roentgen of radiation given to the male parent.

The results, presented in Table 9, show a consistent increase in the regression of outbred offspring on parent for weights (one exception in 21-day weights), attaining significance in two cases. The results for the inbreds appear to be different, with most of the values smaller than those for the outbreds, five of them negative, two of them significantly so. If the genetic variance were purely additive in nature, the regression of offspring on parent would be enhanced for the

TABLE 8

Effects of induced and spontaneous mutations on the within-litter variance of female body weights and age at vaginal opening

Trait	Average cumulative effect			Test for nonlinearity†
	Spontaneous	Induced (/gamete/100r)		
	Homozygous effect	Heterozygous effect	Homozygous effect	
Body weight				
21-day	−0.16 ± 0.18	0.01 ± 0.02	0.19 ± 0.25	P<0.001
42-day	−2.66 ± 2.05	0.03 ± 0.24	−2.13 ± 3.37	P<0.001
69-day	−6.01 ± 4.09	−0.24 ± 0.49	0.93 ± 6.35	P<0.001
Age at vaginal opening	−0.33 ± 0.21	−0.00 ± 0.03	−0.31 ± 0.38	P<0.001

† Test of goodness of fit to the linear model.

TABLE 9

Increase in regression of offspring on parent per roentgen of cumulative exposure

Relationship	Mating system†	Trait‡			
		21-day weight	42-day weight	69-day weight	Age at vaginal opening
sire-son	O	—0.07 ± 0.10	0.04 ± 0.15	0.20 ± 0.12	
sire-son	I	**—0.57 ± 0.18	0.02 ± 0.24	0.04 ± 0.23	
sire-daughter	O	0.14 ± 0.09	*0.15 ± 0.06	0.12 ± 0.07	
sire-daughter	I	*—0.45 ± 0.23	0.04 ± 0.22	—0.10 ± 0.15	
dam-son	O	0.19 ± 0.20	0.39 ± 0.26	**0.61 ± 0.18	
dam-son	I	—0.09 ± 0.22	0.03 ± 0.37	0.11 ± 0.62	
dam-daughter	O	0.30 ± 0.17	0.17 ± 0.11	0.19 ± 0.13	—0.24 ± 0.17
dam-daughter	I	—0.06 ± 0.23	0.11 ± 0.25	0.32 ± 0.25	0.13 ± 0.17

† O = outbred, I = inbred.

‡ Each value has been multiplied by 10⁴.

* P < .05.

** P < .01.

inbred offspring ($R_{op} = .67$). The results indicate the opposite of what would be expected under the additive hypothesis. It is concluded that nonadditive genetic variance is probably being expressed. Real negative genetic covariances between inbred and non-inbred relatives can occur if overdominance is the prevailing kind of gene action. The present results point in this direction.

When the effects of irradiation on the means, within-litter variances, and regression of offspring on parent are considered jointly, the results are most easily interpreted by assuming overdominance of induced mutations affecting body weight (adjusted for size of litter, parity and age of dam). The homozygous effects of these mutations appear to decrease body weight to a greater extent than the heterozygous effects increase it. Thus, selection for increased body weight would be expected to increase the gene frequency of the mutant alleles to some intermediate value less than 0.5. The results of selection for low body weight are less predictable, but would be expected to lead to fixation of either the mutant or the preexisting allele.

The results of the present experiment hold out some hope that X-irradiation could be used in conjunction with artificial selection for the more rapid improvement of domestic animals. An estimate of the cost of such a program in terms of the induced genetic load has also been obtained (TAYLOR and CHAPMAN 1969). Only with a knowledge of the potential gains and their costs can sound decisions be made on the practical value of this approach.

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SUMMARY

Significant effects of cumulative spermatogonial irradiation were observed for

age of vaginal opening and body weights (adjusted for litter size, parity and age of dam). Analyses of the means, the within-litter variances, and the covariances between offspring and parent, indicate that the average heterozygous effects of induced mutations are to increase body weights and decrease age at vaginal opening, while the average homozygous effects are to decrease body weights and to increase age at vaginal opening. Overdominance of induced mutations with respect to these quantitative traits is, therefore, indicated.

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