EFFECTS OF LOW-LEVEL IRRADIATION ON FITNESS AND SKELETAL VARIATION IN AN INBRED MOUSE STRAIN

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TWO problems in mammalian radiation genetics which are of great importance from the point of view of human hazards, and which have been little studied heretofore, are (1) the extent to which a persistent increase in the level of background radiation would be expected to change the fitness of a population, and (2) the genetic effects of low-level irradiation on quantitative variation. The experiment to be described attempted to gain evidence on these points by keeping sublines of the highly inbred C3H strain of mice in a γ radiation field for many generations and comparing the results with those for unirradiated sister sublines. For the work on quantitative variation, continuous and quasi-continuous skeletal characters were used; a preliminary account of this part of the experiment has already been published (SEARLE 1964).

EXPERIMENTAL PROCEDURE

Between March 1957 and September 1958 DR. T. C. CARTER set up the foundation matings for 28 pairs of sublines, all derived from the C3H/HeH strain which had then been sib-mated for about 80 generations. Each pair of sublines originated in a single litter, from which one pair of mice were put in the γ radiation field at the age of 4 to 8 weeks to start the irradiated subline, while another pair was placed at the same time in the adjacent shielded area to start the corresponding control subline. The sublines were kept by an unbranching "parallel line" system, with a single sib-mating in each generation, except that if a particular mating failed to reproduce successfully, it was replaced if possible. Normally three consecutive generations were alive at one time; when the third started to reproduce, the first was killed off. Matings of corresponding control and irradiated sublines were synchronised as far as possible so that sister sublines would pass through the same number of generations. The average generation time was about 80 days.

Irradiated sublines were exposed to 1r Cobalt-60 γ rays for 11 to 17 hours every night, the period lengthening with decay of the source. The dose-rate during exposure varied between 0.0015 and 0.00098r/min, with a mean of 0.0012r/min.

All litters were sexed and classified at birth and weaning, any mouse which diverged from the normal wild-type appearance being kept for genetic tests. Litters of 0 were recorded when a female was pregnant but no live born young were found subsequently. If one member of a subline pair became extinct, then the other member was also discontinued. Three of the original subline pairs are still in existence, having persisted successfully for 25 to 30 generations. In addition, four of the oldest subline pairs were split into two or (in one case) three after 10 to 15 generations to try to make sure that one branch at least would continue. Two of these were split again after 19 generations. Of these branches two still continue.

In order to disentangle somatic from genetic effects and to find how many dominant and recessive lethal mutations are being induced, 17 pairs of mice were transferred from the irradiation to the control area after 20 or more generations, being derived from nine different sublines in all. They were sib-mated and both sib- and nonsib-matings made of their offspring, F_1 females being dissected on the 14th day of gestation and counts made of numbers of corpora lutea, live

FIGURE 1.—Pedigree of the two pairs of C3H sublines (1 and 6) from which skeletal material was collected for subline divergence studies. Numbers of generations between matings are shown in parenthesis, while broken lines represent generations of irradiation.

and dead implants to find the extent of embryonic lethality. Similar sib- and nonsib-matings and dissections were made from offspring of control and irradiated sublines which had not been transferred, for comparison.

Two particular subline pairs, 1 and 6, were used for investigating the genetic effects of the irradiation on continuous and quasi-continuous variation, the experimental design being shown in Figure 1. Litters were removed from the control and irradiated sides of subline 1 during the ninth and tenth generation after foundation and from subline 6 during the eighth generation. Numbers were expanded over two to three generations. Resultant offspring were then processed by the papain method, mostly at the age of 2 to 3 months, and the resultant disarticulated skeletons classified with respect to 23 skeletal variants. Most of these variants were first described by GRÜNEBERG and coworkers in their series of papers on the mouse skeleton and relevant information has been summarized by BERRY and SEARLE (1963). A few variants not previously described are illustrated in the present paper. The variants were:

1. Bent-nose (Figure 2).
2. Nasal fused to skull (Figure 2). One or both nasals become fused proximally to the underlying ethmoid bone, thus remaining in the skull instead of becoming dislodged as a result of the enzymatic maceration.
3. Interfrontal present.
4. Parted frontals.
5. Frontal foramen absent. The frontal foramen may be absent or very small on one side of the skull, though normally large in this strain. It was only classified as present when medium or large.
6. Fenestra flocculi of the periotic bone absent.
7. Jugal not fused to skull (Figure 3). The jugal bone is normally fused to the squamosal to form the zygoma, but a suture may remain between them so that the jugal becomes detached and lost during the papain process.
8. Foramen ovale double.
9. Foramen hypoglossi double.
10. Foramen sphenoidale medium absent.
11. Presphenoid without preoptic sutures.
12. Presphenoid with metoptic roots.

13. Alveolar bone absorption. The alveoli around the molar teeth of the upper jaw may disappear almost completely on one or both sides of the skull (Figure 3). This variant was only classified as present when there was heavy absorption, with loss of the sockets for the second and third molars at least.
14. Accessory mental foramen.
15. Dyssymphysis posterior of the axis.
16. Processus spinosus on the third cervical vertebra (C.III). This was counted as present even when very small.
17. Processus spinosus on the second thoracic vertebra (Th.II).
18. Tuberculum anterius of C.VI dystopic, being partly or wholly shifted on to C.V or C.VII.
19. C.VII with foramen transversarium. Normally these foramina are only found on the first six cervical vertebrae.
20. Sacralization of the sixth lumbar vertebra (L.VI) on one or both sides.
21. Sacral arches not fused. Normally the neural arches of the first two or three sacral vertebrae fuse together in this strain.
22. Foramen acetabuli perforans in the pelvic girdle.
23. Humerus with fossa olecrani perforata.

In addition, the following characters were measured with a travelling microscope:—

1. Length of left ilium, from distal end to edge of acetabulum.
2. Length of left humerus, without its epiphysis.
3. Crown length of left lower first molar tooth.
4. Bucco-lingual width of crown of lower third molar tooth (left or right at random).

DEOL, GRÜNEBERG, SEARLE, and TRUSLOVE (1957) have shown that if sublines of the same inbred strain are compared with respect to the frequencies of quasi-continuous skeletal variants they are found to diverge from each other more and more the further they are apart in terms of generations. They concluded that this subline differentiation was due to the action of spon-

FIGURE 2.—Dorsal view of mouse skull with bent-nose to left and left nasal fused to skull.

FIGURE 3.—Ventral view of mouse skull showing alveolar bone absorption in the right tooth row and nonfusion of the right jugal.

taneous mutations affecting the frequencies of particular variants and becoming incorporated into particular sublines. The rate of subline differentiation is thus a measure of the mutation rate to the genes concerned, which are essentially the same as the "polygenes" of continuous variation. GRÜNEBERG therefore suggested that a comparison of the rate of subline differentiation with and without the added factor of irradiation should make it possible to estimate the radiation-induced mutation rate for this type of variation. This is what was attempted in the present experiment.

RESULTS

The following indicators of fitness were investigated in control and irradiated lines: (1) Incidence of sterility and lowered fertility. Sterility was used in a wide sense to include all matings in which no live offspring were produced, including those in which this was due to death of one or other partner. (2) Neonatal three-litter productivity. The analysis excluded sterile matings but included all others which did or could have had three litters if not ceasing to breed. Overall production of newborn young could not be used as a measure since matings were usually ended when F_2 offspring were breeding, which was often before the matings themselves had ceased to breed. (3) Litter-size at birth, analysed separately for first, second, third, fourth and fifth plus sixth litters. Litters of 0 were excluded. (4) Postnatal mortality between birth and weaning age, excluding losses of whole litters. (5) Incidence of litters of 0 at birth and at weaning. These were used as indicators of maternal insufficiency. (6) Embryonic lethality in various types of mating, to see what proportion could be attributed to somatic and to various possible genetic effects of the irradiation.

The first 24 generations were analyzed and the following results obtained.

Sterility and lowered fertility: Over 90 percent of all matings (Table 1) in both series successfully produced three litters. The proportion of sterile matings and of matings with less than three litters was slightly higher in the irradiated series, but the differences are not significant, neither is there any tendency for the proportion affected to increase in later generations with a greater cumulative dose of radiation.

Productivity: Productivity tended to decline in both control and irradiated sublines (Figure 4) although there was some recovery after low values in the 11th and 12th generations. Mean productivity at birth in irradiated sublines was 4.2

TABLE 1

Comparison of sterility and lowered fertility in control and irradiated matings

	Generations	Classifiable matings	Without offspring		With 1 to 2 litters		With 3 or more litters	
			Number	Percent	Number	Percent	Number	Percent
Control	1-12	248	7	2.8	10	4.0	231	93.1
	13-24	168	4	2.4	7	4.2	157	93.5
	All	416	11	2.6	17	4.1	388	93.3
Irradiated	1-12	240	6	2.5	15	6.3	219	91.3
	13-24	158	3	1.9	10	6.3	145	91.8
	All	398	9	2.3	25	6.3	364	91.5

FIGURE 4.—A comparison of three-litter productivity in control and irradiated sublines over 24 generations.

percent less than in control sublines, but Figure 4 shows that the difference was greatest in generations 9 to 14, declining sharply thereafter. Three-litter production of weanlings was actually 1.6 percent higher in irradiated sublines than in controls.

Litter size at birth: Comparison of control and irradiated trends with respect to litter size gives a clearer picture of what is happening (Figures 5 and 6). There is very little difference between the two series with respect to first and second

FIGURE 5.—Litter-size trends over 24 generations for first (top), second and third litters of control and irradiated C3H sublines.

FIGURE 6.—Litter-size trends for fourth litters (top) and fifth plus sixth litters.

TABLE 2

A comparison of numbers born in sister control and irradiated C3H sublines with respect to first three litters, excluding litters of zero

Litters	Generation	Control total	Irradiated total	Percent difference	t	P
First	1-12	1410	1401	— 0.6	0.22	>0.80
	13-24	663	647	— 2.4	0.55	>0.50
Second	1-12	1383	1345	— 2.7	0.94	>0.30
	13-24	722	704	— 2.5	0.57	>0.50
Third	1-12	1305	1211	— 7.2	2.16	<0.05
	13-24	656	587	—10.5	2.19	<0.05

litters (Table 2), but in third and later irradiated litters the litter size tends to diverge more and more from the control figures, until it is consistently below them for fifth and sixth generations combined (Figure 6). These last litter sizes are based on rather small numbers as far as irradiated matings are concerned, since most of them became sterile before producing a fifth litter.

That this phenomenon is due to somatic rather than genetic effects of radiation is indicated by the fact that: (1) only later litters are affected; (2) the effect is apparent even in very early generations, apart from the first, in which individuals were not mated up in the irradiation field until the age of at least 4 weeks; (3) matings in irradiated sublines tended to stop breeding much sooner than control matings; and (4) there was a similar but much more marked effect in some matings put on 2r per night, with only one out of 66 producing more than three litters. In view of the known very high radiosensitivity of the dictyate oocyte in young female mice (OAKBERG 1962) the effect on litter size can be confidently regarded as mainly due to the depletion of oocyte stocks in the ovary due to killing, leading to reduction in numbers ovulated and the early onset of sterility.

Postnatal mortality: Table 3, based on 11,000 offspring in the control sublines and over 8,000 in the irradiated ones, shows that postnatal mortality is lower in the irradiated series for all parities and for both early and later generations. There was significant heterogeneity with respect to birth order, but analysis by COCH-

TABLE 3

Percentage mortality between birth and weaning in offspring of control and irradiated matings, omitting litters with no survivors

Litter order	Control offspring		Irradiated offspring	
	Generations 1 to 12	Generations 13 to 24	Generations 1 to 12	Generations 13 to 24
1	6.1	8.7	2.8	7.2
2	2.8	9.2	1.9	6.9
3	4.2	8.8	2.6	6.4
4	4.9	13.0	2.2	5.1
5 + 6	5.2	12.7	3.6	8.7
All	4.65	10.54	2.46	6.69

TABLE 4

Incidence of litters of 0 at birth and at weaning in offspring of control and irradiated matings

Litter order	Birth		Weaning	
	Control matings	Irradiated matings	Control matings	Irradiated matings
1	1.7	0.8	5.04	2.76
2	1.7	0.8	7.02	2.12
3	2.9	1.1	6.95	5.00
4	3.9	5.7	6.67	6.42
5-6	6.7	9.7	9.46	8.81
	2.39 $\pm$ 0.44		6.30 $\pm$ 0.70	
	0.87 $\pm$ 0.27		3.25 $\pm$ 0.53	
	5.61 $\pm$ 0.77		8.31 $\pm$ 0.95	
	7.19 $\pm$ 1.21		7.31 $\pm$ 1.26	

RAN's (1954) method showed highly significant differences between control and irradiated sublines for both groups of generations ($u = 6.06$ for 1-12 and 4.72 for 13-24, where u is the unit normal deviate). In both series, postnatal mortality is also significantly higher in later than in earlier generations, COCHRAN's u being 12.04 for controls and 9.57 in the irradiated series. Mortality in irradiated sublines was 53 percent of controls in the first 12 generations and 63 percent in the second 12. The effect shows up clearly in the first and second litters, so cannot be just the result of decreased litter size in the irradiated series, since there was very little difference between the two series in the sizes of first and second litters.

Litters of 0: As Table 4 shows, there is an interesting situation with respect to losses of whole litters. Both at birth and at weaning there were significantly fewer losses in the irradiated series than in controls with respect to litters 1 to 3, but there were more losses at birth in the irradiated series with respect to litters 4 to 6, although the difference is not significant. Presumably the changed situation in later litters is connected with the litter-size decrease in irradiated matings, making it more likely none would survive. In both control and irradiated sublines there was a tendency for more neonatal and postnatal losses in later generations, which was probably at least partly the result of increasing inbreeding degeneration.

Embryonic lethality: The pairs providing offspring for matings to determine the extent of embryonic lethality belonged to the 20th or later generations from the foundation of the sublines concerned; the offspring were mated up at 10-11 weeks of age and females dissected on the 14th day of gestation.

Table 5 shows that survival to 14 days was decidedly less in the irradiated subline matings than in controls and in sib-matings than in nonsib, suggesting the action of induced recessive lethal mutations. Since the results for corresponding matings of irradiated sublines in control and in radiation areas do not differ significantly, it can be concluded that somatic effects of irradiation were not important here. Pooling the two sets of results, therefore, we obtain the following estimates of survival to 14 days:

	Control	Irradiated
Sib-matings	0.782 $\pm$ 0.016	0.707 $\pm$ 0.014
Nonsib-matings	0.805 $\pm$ 0.016	0.761 $\pm$ 0.013

TABLE 5

A comparison of embryonic survival to 14 days in sib and non-sib matings from control and irradiated sublines, and from members of irradiated sublines transferred to the control area the previous generation

Sublines	Type of mating	Females opened	Corpora lutea	Total implants	Live embryos	Ratio (live embryo)/ (corpus luteum)
Control	sib	68	666	600	521	0.782 ± 0.016
	nonsib	66	629	571	506	0.805 ± 0.016
Irradiated	sib	48	445	388	314	0.706 ± 0.022
	nonsib	65	614	545	461	0.751 ± 0.017
Irradiated in control	sib	59	557	493	394	0.707 ± 0.019
	nonsib	55	520	474	402	0.773 ± 0.018

The differences in survival between control and irradiated sublines are significant both for sib- and for nonsib-matings, the difference between sib- and nonsib-mating survival with respect to irradiated sublines is also significant. From these figures we can estimate that embryonic survival in sib-matings was 97.2 percent of that in nonsib ones for controls and 92.9 percent for the irradiated series.

The average numbers of corpora lutea in irradiated and unirradiated females were very similar, showing little sign of any direct action of γ irradiation on ovulation rate with respect to these first litters. A comparison of the proportion of implantations surviving to 14 days with different numbers of implantations in the control series showed little evidence for intrauterine compensatory tendencies which might mask lethal action (CARTER and LYON 1961).

Skeletal variation. Quasi-continuous characters: In all, 2004 mice were classified with respect to skeletal variants, the distribution by sex and subline being as follows:

	Subline 1		Subline 6	
	Control	Irradiated	Control	Irradiated
Male	289	206	233	232
Female	325	207	238	274
Total	614	413	471	506

Table 6 shows the incidence of the 23 skeletal variants in the four groups. Where the variant has a lateral effect, the frequency is expressed as percentage of sides affected rather than percentage of mice. Although the frequencies are generally fairly similar there are some marked differences, for instance with respect to absence of fenestra flocculi (No. 6) and presence of a spinous process on the second thoracic vertebra (No. 17). It was decided to estimate the extent of divergence between sublines by the method used by GREWAL (1962) and BERRY (1963) on similar material. The percentage incidences p , were therefore transformed into angular values θ , where $\theta = \sin^{-1} (1 - 2p)$, measured in radians. The measure of divergence between samples 1 and 2 with respect to a particular character was taken as

$$(\theta_1 - \theta_2)^2 - (1/n_1 + 1/n_2)$$

TABLE 6

Percentage incidence of skeletal variants in control and irradiated lines

Character	Subline 1		Subline 6	
	Control	Irradiated	Control	Irradiated
1. Bent-nose	35.3	28.4	28.0	28.3
2. Nasal fused to skull	52.0	64.3	50.9	46.8
3. Interfrontal present	13.5	23.3	21.2	17.6
4. Parted frontals	11.5	15.5	11.1	9.8
5. Frontal foramen absent	16.1	18.5	19.4	19.5
6. Fenestra flocculi absent	2.4	11.3	20.2	13.3
7. Jugal not fused to skull	4.1	9.6	9.5	8.0
8. Foramen ovale double	3.7	5.2	4.3	2.3
9. Foramen hypoglossi double	45.0	36.6	36.8	30.9
10. No foramen sphenoidale medium	17.4	15.3	13.7	20.8
11. Presphenoid: no preoptic sutures	4.2	8.7	11.0	7.7
12. Presphenoid: metoptic roots	14.9	16.0	21.9	22.3
13. Alveolar bone absorption	27.3	39.7	52.2	49.1
14. Accessory mental foramen	21.4	20.4	24.6	20.7
15. Dyssymphysis posterior of axis	18.8	17.1	24.4	14.4
16. Proc. spin. of C.III present	35.5	44.4	31.7	25.8
17. Proc. spin. of Th.II present	37.7	61.9	61.3	63.0
18. Tuberculum anterius dystopic	1.8	0.9	1.2	1.0
19. For. trans. on C.VII	16.8	13.1	16.4	12.7
20. Sacralization of L.VI	23.0	34.7	36.1	28.5
21. Sacral arches not fused	2.8	4.5	9.6	8.2
22. Foramen acetabuli perforans	7.2	8.5	6.4	15.7
23. Fossa olecrani perforata	40.6	44.8	57.0	62.2
Total classified	614	413	471	506

where n_1 and n_2 are the numbers in the two samples. This measure has variance V equal to $1/n_1 + 1/n_2$. The measure of divergence is distributed as $V(\chi^2 - 1)$ and will therefore be significant at the 1 percent level if it is greater than $5.64V$ and at the 0.1 percent level if greater than $9.83V$. These two levels of significance were used in the present analysis.

All possible comparisons between the different sublines were made and Table 7 shows that for only six variants no significant differences at the 1 percent or higher levels were found. Surprisingly enough, the largest number of significant differences occurred between the two control sublines; in fact, the three highest numbers of differences all involved control subline 1.

A mean measure of divergence for each comparison was obtained by summing all the divergences and dividing by the number of variants (Table 8); all measures differed significantly from zero. The average rate of divergence per generation was then calculated. Table 8 shows that the average rate of divergence between the two control sublines was 2.5 times that between the two irradiated sublines. Of the 27 generations separating the two samples of mice examined in the irradiated series, 18 were with and nine without irradiation. If we assumed that the rate of divergence in the unirradiated generations was the same as that found between the controls (namely 0.0022) then we would have to conclude that very

TABLE 7

Differences between the incidence of skeletal variants in Control (C.) and Irradiated (I.) lineages which are significant at the 1 percent (+) and the 0.1 percent (++) probability levels. Variants showing no significant differences are omitted

Character	Lineages compared					
	C.1-C.6	I.1-I.6	C.1-I.1	C.6-I.6	C.1-I.6	C.6-I.1
1. Bent-nose	+					
2. Nasal fused		++	++			++
3. Interfrontal	++		++			
4. Parted frontals		+				
6. No fenestra flocculi	++		++	+	++	++
7. Jugal not fused	++		++		+	
9. For. hypoglossi double	+		+		++	
11. No preoptic sutures	++		+			
12. Metoptic roots	+				+	
13. Alveolar bone absorption	++	+	++		++	++
15. Axis dyssymphysis				++		+
16. P.s. of C.III		++	+		++	++
17. P.s. of Th.II	++		++		++	
20. Sacralization of L.VI	++		++			
21. No sacral arch fusions	++				++	+
22. For. acet. perforans		++		++	++	
23. Fossa olecrani perforata	++	++			++	++
Total	12	6	10	4	10	7

little divergence at all happened during the irradiated generations, since $0.0251 - (9 \times 0.0022) = 0.0053$. It would appear, however, that most of the divergence between the control sublines occurred in subline 1, since the rates of divergence between control subline 6 and each of the irradiated sublines are very low.

Evidence for an abnormally high rate of subline divergence within control subline 1 is reinforced when an attempt is made to determine, through inspection of Tables 6 and 7, the least number of mutational steps which could account for the major differences found (assuming independent gene action on different variants) and in which section of the pedigree the event took place. Table 9

TABLE 8

Measures of divergence between control (C.) and irradiated (I.) lines of C3H mice, derived from incidence of 23 skeletal variants

Comparison	Generations apart	Mean measure of divergence per variant	Standard deviation	Mean divergence/standard deviation	Average rate of divergence per generation
C.1 and C.6	27	0.0598	0.0062	9.64	0.0022
I.1 and I.6	27	0.0251	0.0044	5.73	0.0009
C.1 and I.1	25	0.0343	0.0049	7.02	0.0014
C.6 and I.6	21	0.0119	0.0029	4.08	0.0006
C.1 and I.6	27	0.0539	0.0058	9.28	0.0020
C.6 and I.1	27	0.0163	0.0036	4.56	0.0006

TABLE 9

Probable numbers and positions of mutational events markedly changing the frequency of particular skeletal variants (indicated by reference number) which occurred within or between the control and irradiated lines

Position	Mutating variants	Total number
Between sublines 1 and 6	12,13,21,23	4
Within C.1	1,3,6,7,9,11,13*,17	7 or 8
Within C.6	6,15	2
Within I.1	2,4,13*,16	3 or 4
Within I.6	10,16,22	3
Total		20

* Positions equally likely.

shows that of the 20 mutational events necessary, seven or eight must have occurred in control subline 1. Many of the divergences between this subline and the others are very highly significant and two characters (alveolar bone absorption and absence of fenestra flocculi) account for nearly half the total divergence.

There were two groups of mice from control subline 1 which were examined for skeletal variation (Figure 1) individuals of one group being separated by seven generations from individuals of the other. Measures of divergence between these two groups with respect to the skeletal variants were worked out, in case they threw light on the origin of the high rate of differentiation in subline 1 controls. Results were as follows:

Mean divergence per variant	Mean divergence/sp	Rate of divergence per generation
0.0029	1.59	0.0004

The rate of divergence is low, with the mean divergence not significantly different from zero, although two individual measures of divergence (for interfrontal and fenestra flocculi) are significant at the 5 percent level of probability. The results indicate that the source of the abnormally high divergence must have been in some earlier generations.

Skeletal variation. Metrical characters: Bone measurements were made on 1274 mice in all, belonging to the first five litters. Sexes were pooled in the analysis of tooth and humerus measurements, but there seemed to be a marked sex difference with respect to the ilium and only the data on females were used.

Table 10 shows that significant differences between the sublines have developed, the most obvious being those for the size of lower third molar teeth. If differences are put in terms of their standard errors and all possible comparisons are made (Table 11) it is seen that, as with quasi-continuous characters, the average rate of divergence per generation between the two control lines is greater than between the two irradiated. The total divergence between C.1 and other sublines is again greater than between C.6 and other sublines, although the

TABLE 10

Mean values of metrical characters in control and irradiated sublimes, with standard errors

	Subline 1		Subline 6	
	Control	Irradiated	Control	Irradiated
Female left ilium (mm)	10.20 $\pm$ 0.03	10.27 $\pm$ 0.04	10.30 $\pm$ 0.05	10.17 $\pm$ 0.04
Left humerus (mm)	10.61 $\pm$ 0.02	10.66 $\pm$ 0.02	10.77 $\pm$ 0.02	10.69 $\pm$ 0.02
Left lower first molar (mm/100)	151.73 $\pm$ 0.13	151.10 $\pm$ 0.17	151.12 $\pm$ 0.15	150.70 $\pm$ 0.14
Lower third molar (mm/100)	57.66 $\pm$ 0.16	57.05 $\pm$ 0.19	55.91 $\pm$ 0.18	54.62 $\pm$ 0.19

TABLE 11

Differences between sublimes with respect to metrical characters, estimated in terms of their standard errors

	Comparisons					
	C.1-C.6	I.1-I.6	C.1-I.1	C.6-I.6	C.1-I.6	C.6-I.1
Female left ilium	1.7	1.9	1.4	2.1	0.7	0.4
Left humerus	5.9	1.0	2.3	2.7	3.4	3.5
Left lower first molar	3.1	1.9	3.0	2.1	5.3	0.1
Lower third molar	7.3	9.1	2.5	4.9	12.3	4.4
Mean difference	4.50	3.48	2.30	2.95	5.43	2.10
Number of generations	27	27	25	21	27	27
Mean divergence/generation	0.167	0.129	0.092	0.140	0.201	0.078

effect is less marked than before. With lower third molar size, there are signs that some divergence between subline pairs 1 and 6 had developed before the experimental period.

DISCUSSION

Fitness: The 80r/generation of γ irradiation to which a number of sublimes have been subjected for the past 6 to 7 years has had the expected many-sided effect on fitness, an effect which is partly somatic and partly genetic, partly *via* the maternal genotype and partly on the individual direct.

There seems little doubt that the marked decrease in litter size of the third and later litters was very largely somatic in origin, owing to early killing of oocytes and resultant depletion of stocks. OAKBERG (1962) showed that early oocyte stages in 10-day old mice were particularly sensitive to the killing effect of radiation, with LD₅₀ of 8.4r for stage I oocytes after γ irradiation at 2.85r/min. Chronic γ irradiation had a less extreme effect; nevertheless there was only 13 percent survival of oocytes after 26r of γ irradiation between days 10 and 12 at 0.009r/min. Results of the present experiment show that there must still be a fairly severe effect at a dose rate only about one-eighth of this. L. B. RUSSELL, STELZNER, and W. L. RUSSELL (1959) showed that oocyte killing by chronic γ irradiation has no appreciable effect on first litters, but leads later to a steep decline in production ending in sterility. 86r γ rays at various low dose-rates had

no significant effect on fertility although 171r did. However, females were at least 6 weeks old when treatment began, so the most sensitive period was not at risk. The early onset of the sensitive period also explains why there was little sign of any decrease in litter size for the first generation of the present experiment, since foundation pairs were at least 4 weeks old when put in the irradiation field.

BROWN, KRISSE, PACE, and DEBOER (1964) found that Swiss albino female mice kept on 2r/day continuous (apart from 1 hr/day) γ irradiation from conception became sterile after 25 weeks of postnatal life. C3H mice in our laboratory which were kept on 2r/day had their last litter when 15 weeks old on the average, (omitting those completely sterile), usually producing two or three litters and very seldom four. This suggests that C3H oocytes may be more radiosensitive than those of Swiss albinos, although dose-rate and fractionation differences between the experimental treatments make them not exactly comparable. But there seems little doubt that there are major differences between strains in this respect, since C57BL sublines very rapidly become extinct with 1r/day of γ irradiation owing to early sterility (DR. J. GODFREY, personal communication).

Irradiation is also known to have another somatic effect on the ovary, for it leads to a significant increase in number of eggs ovulated if the dose is delivered shortly before fertilization (L. B. RUSSELL and MAJOR 1953). In the present experiment, however, numbers of corpora lutea in irradiated and unirradiated pregnant females derived from irradiated sublines were very similar to each other. Thus the effect seems negligible at these low radiation levels.

The general decline in productivity and litter size, even of first litters, which took place in later generations of both control and irradiated mice, was presumably the result of continuing inbreeding degeneration. This was much more severe in these unbranching sublines, with minimal opportunities for artificial selection, than in the main C3H stock, where grading ensures that productivity remains roughly constant from generation to generation. Thus at a time when average litter size, based on four-litter totals, was 7.2 in the main stock, it was only 5.6 in control sublines of this experiment.

Investigation of embryonic survival to 14 days in control and irradiated sublines, after sib and nonsib-matings, provided evidence for the induction of recessive and other lethal mutations. There was also a slight (nonsignificant) litter-size decrease in even the first two litters from irradiated matings (Table 2). Using the calculations of HALDANE (1936) and LYON (1959) we can estimate the frequency of intercrosses for a lethal in control and irradiated series from comparisons of survival in sib- and nonsib-matings, and can derive the overall mutation rates to recessive lethals. Thus the following expression was used to derive the frequency of intercross matings m from the relative survival s in sib- as compared with nonsib-matings:

$$e^{-m}(1 + \frac{3}{4}m + (3/4)^2 \frac{m^2}{2!} + \dots) = s$$

The value of s was 0.972 in controls and 0.929 in the irradiated series. The re-

lationship between m and the mutation rate depends on the method of maintaining the strains: where an intercross leaves as many offspring as the other two types of mating in the next generation (as in the present scheme) then the frequency of intercross matings is 6 times the overall mutation rate to recessive lethals. Results were as follows:

	Percent intercross mating	Mutation rate	Induced rate	Induced rate/r
Control	11.77	1.96×10^{-2}		
Irradiated	29.42	4.90×10^{-2}	2.94×10^{-2}	1.84×10^{-4}

In calculating the induced mutation rate per roentgen the dose per generation has been taken as 160r, since mutations could be induced in all germ-cell stages of either sex, each being exposed to 80r. An experiment on a much larger scale would be needed to obtain a really accurate estimate of the rate of induction of recessive lethals in these circumstances, but the figure of 1.8×10^{-4} is similar to the estimates of LÜNING (1964) and LYON, PHILLIPS, and SEARLE (1964) which suggested a rate of about 2×10^{-4} /gamete/r with spermatogonial X irradiation at high intensity. A lower mutation rate would be expected at lower dose rate when premeiotic germ cells were irradiated, but not with treatment of post-meiotic cells (RUSSELL, RUSSELL, and KELLY 1958) which were also at risk in the present experiment. The overall spontaneous mutation rate to recessive lethals estimated from the present data is lower than the figure of 0.1 per gamete obtained by LYON (1959). But LYON pointed out that her estimate was an upper limit to the rate. The difference is probably connected with the fact that LYON's nonsib-matings were between members of different strains, while in the present experiment they were all within the same strain, though involving different sublines.

When analyzing the results of nonsib-matings it is best to consider the three types of mating separately, namely controls, irradiated and irradiated in control area. Survival in the irradiated group was 93.3 percent of controls, but was 96.0 percent of controls for the irradiated group kept in the control area. Thus a mortality of 2.7 percent can be attributed to direct lethal effects of the radiation on embryos or to the induction of dominant lethal mutations, which would not be inherited. The latter seems more probable, since RUSSELL, BADGETT, and SAYLORS (1959) found that the litter size of females continuously irradiated with 171r of γ irradiation for the first 14 days of pregnancy was perfectly normal; the dose in the present experiment would be only 1/12 of this. PHILLIPS and SEARLE (1964) have shown that the yield of dominant lethals is negligible even after 1200r of chronic γ irradiation of mouse spermatogonia at 100r/week, owing to a dose-rate effect. But postmeiotic germ cells in the male would be subjected to a dose of about 30r of γ rays, from which an appreciable yield of dominant lethal mutations might be expected, owing to the probable absence of a dose-rate effect and the much higher sensitivity of these later germ-cell stages. The magnitude of the effect in females is more doubtful, although it is known that the LD₅₀ with respect to dominant lethal induction is about 600r of X rays for the mature dictyate oocyte (RUSSELL and SPEAR 1955; EDWARDS and SEARLE 1963).

The remaining excess mortality of 4.0 percent is attributable to the induction of heritable mutations tending to have a lethal effect, for example translocations, inversions, or point mutations with a detrimental effect in the heterozygote. Translocations seem to be excluded by the fact that none of the pairs transferred from irradiated sublines to the control area showed semisterility and none of the pregnant females which were dissected showed the embryonic lethality of around 50 percent which would be expected with heterozygosity for a translocation. The frequency of inversions seems to be very low in mammals, so it seems likely that this extra mortality was mainly due to heterozygous effects of recessive lethal and other mutations.

The fitness of a population depends to an important extent on maternal characteristics, especially the ability to produce liveborn young and rear them successfully to weaning age. In these respects, members of C3H irradiated sublines showed signs of being superior to controls. Complete loss of a litter is usually due to maternal insufficiency rather than to the action of individual lethals or sub-lethals, and such loss was significantly more frequent in control than in irradiated matings (Table 4) if the first three litters were compared. The marked reduction in mortality between birth and weaning which occurred in the offspring of irradiated matings as compared with controls (Table 3) may also be partly due to maternal influences. LÜNING (1960) also reported a lower rate of death before weaning in F_1 offspring of males of the highly inbred CBA strain, which had been given 276r of acute X irradiation and mated after the sterile period to CBA females. In later generations, however, the rate of death was significantly higher in offspring of those females of the irradiated series which had lost one or more litters, as compared with the corresponding controls (LÜNING 1963). Slightly more litters were also completely lost in the irradiated series than in the controls. It should be noted, however, that LÜNING's populations of control and experimental CBA mice were propagated by random mating, so as to accumulate recessive mutations; in the present experiment there was sib-mating throughout.

One possible explanation of findings in the present experiment might lie in the phenomenon of overdominance, as discussed recently by WALLACE (1963). The increasingly severe postnatal effects of inbreeding degeneration might be partially counteracted in some irradiated sublines by the induction of mutations which, in heterozygous condition, gave an increase in vigour as compared with the previous homozygote. If so, one would have the paradoxical situation that mutations in heterozygous condition were apparently having a prenatal detrimental effect (increasing embryonic lethality) but a postnatal beneficial effect. Several other possible explanations of these postnatal findings cannot yet be discounted however, for instance that the γ radiation is unfavourably affecting pathogenic organisms in the mice (such as those causing juvenile diarrhoea), or that there is some subtle difference between control and radiation environments.

Skeletal variation: Results of the skeletal studies are also difficult to interpret at this stage. There was abundant evidence that subline differentiation was occurring both with respect to continuous and quasi-continuous characters. The average rate of divergence per character varied between 0.0006 and 0.0022 per genera-

tion for different subline comparisons with the quasi-continuous variants, which is somewhat below GREWAL's (1962) figure of 0.003 per generation for different sublines of the C57BL strain, in the absence of irradiation. However, the estimate of 20 mutations for the 23 variants in a total of 40 generations (excluding the generations of expansion) would mean an average of 0.022 mutations per variant per generation, which is about double that reported by DEOL *et al.* (1957) in C57BL sublines. GREWAL considered the C57BL rate of one mutation per 100 generations "astonishingly high" when compared with specific locus data and discussed various possible explanations, including the segregation of residual heterozygosis masquerading as mutation. Certainly there is some evidence for this phenomenon in the present experiment, since four "mutations" seem to have occurred between founder members of the two subline pairs, although they are only four generations apart. Some residual heterozygosis may also of course have persisted after subline formation and become fixed later within a particular subline.

One of the metrical characters, namely length of ilium in females, was also used by BAILEY (1959) in his study on rates of subline divergence in the highly inbred strains C57BL/6 and BALB/cAn. His measure of divergence was somewhat different from that used in the present experiment, but he estimated that it would take an average of nine generations in strain BALB and 30 generations in strain C57BL for measurements to become significantly different at the 5 per cent level. The data of Table 10 suggest that the appropriate figure for the C3H strain is similar to that for C57BL. The rate of divergence of C3H sublines with respect to lower third molar size is much more rapid, but, as SEARLE (1960) has emphasised, the mouse third molar is really a vestigial structure which confers no selective advantage by its presence; homeostatic control has therefore been relaxed so that size of the tooth might be expected to vary widely and to be greatly affected by mutation.

For both continuous and quasi-continuous characters the rate of divergence was unexpectedly found to be greater between the two control sublines than between the two irradiated ones. The simple explanation of this finding, namely that the polygenic mutation rate in the presence of irradiation is really lower than in its absence, can be dismissed as highly improbable in view of our general knowledge of radiation genetics and the findings of BUZZATI-TRAVERSO (1960), SCOSSIROLI and SCOSSIROLI (1959), YAMADA and KITAGAWA (1959) on the induction of polygenic mutations in *Drosophila*. We are left with two main possibilities: either this finding is due to some improbable event or events, or it is an example of stabilizing selection. It might be supposed that under the extra stress to which irradiated sublines were subjected selection against extreme variants would be more intense, so that mutations changing the frequency of variants would have less chance of becoming incorporated into the genome. One piece of evidence in favour of this hypothesis is that the only metrical character showing the expected greater divergence between irradiated sublines is size of lower third molar, and it is precisely this character which would be least affected by stabilizing selection, because of the relaxation of homeostatic control. On the other hand, it seems very

doubtful whether the extra stress involved in the low-level irradiation (especially with respect to the first two litters, from which the next generation was usually derived) would be sufficient to account for the intensity of selection necessary to affect these minor variants, which themselves have no appreciable effect on fitness.

The other explanation, of a freak event, is strongly favoured by the way in which the divergence between control sublines has mainly taken place within subline 1 rather than equally within sublines 1 and 6. This is shown by the fact that, for quasi-continuous characters, the two comparisons of subline divergence which involve subline 1 and not 6 both show much higher rates than the two involving subline 6 and not 1 (Table 8); moreover, the probable number of mutational events in control subline 1 is seven or eight, but only two in control subline 6 (Table 9). The recent rate of divergence within control subline 1 has been low, however, so the events concerned must have been concentrated in earlier generations. The results suggest that a "major" polygenic mutation may have occurred in C.1, simultaneously affecting the frequencies of several variants, or alternatively that some residual heterozygosis has reached fixation in this line alone. TRUSLOVE (1961) has investigated the extent to which presence or absence of particular variants is correlated, as would occur if they were being affected by the same mutation. She found relatively few examples of this, some of those she did find being connected with birth weight. The characters TRUSLOVE tested did not include some showing considerable divergence in the present experiment (e.g., fenestra flocculi and alveolar absorption) so it is clearly necessary to investigate the possibility of correlation further.

On the assumption that the main results with respect to C.1 are unrepresentative, so that the recent rate of divergence in the subline and the apparent rate in C.6 would conform more closely to the normal pattern, we find that the data would be in agreement with the hypothesis that the rate of subline divergence in the irradiated sublines is normally about 2.5 times that in the controls. This would be in line with expectation on the basis of specific locus findings (assuming 80r of chronic γ irradiation per generation, delivered in part to postmeiotic germ-cell stages) if stabilizing selection is having a negligible effect and subline differentiation rate can really be equated with polygenic mutation rate. YAMADA and KITAGAWA (1959) concluded that the mutability of polygenic systems involving bristle numbers in *Drosophila* was substantially of the same magnitude as that of major genes.

In conclusion, it can be seen that the work on the effects of low-level irradiation of fitness of inbred mice, which has involved an analysis of data from many sublines, has on the whole given clear-cut results, showing that even at this very low dose rate somatic and genetic effects of irradiation are fairly extensive. But the study of continuous and quasi-continuous skeletal variation, in which data from two subline pairs only were analyzed, has not yet produced a clear picture of the effects of radiation on quantitative variation, and further analysis is necessary. In any future major investigation of the effect of radiation on subline differentiation, it will obviously be wise to have a number of control and irradiated

sublines for comparison, so that anomalous results in one of them can be more easily recognised as such.

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SUMMARY

Sublines of the highly inbred strain C3H/HeH have been kept in a Co^{60} γ radiation field for many generations, receiving 1r per night and about 80r per generation. Data pertaining to fitness have been analyzed for the first 24 generations. Litter size in irradiated sublines was only slightly below controls with respect to first and second litters, but from the third to the sixth litters it fell progressively in irradiated sublines, with an associated early onset of sterility. These findings were attributed to the extreme sensitivity to killing of young dictyate oocytes leading to significant depletion of stocks even at the low dose rate of 0.001-0.0015r/min. The incidence of complete sterility was not increased in irradiated lines, however. Embryonic survival to 14 days was significantly decreased in both sib- and nonsib-matings derived from irradiated sublines, when compared with controls; the extra load of recessive lethal mutations induced by the irradiation of all germ-cell stages was estimated to be 1.8×10^{-4} /gamete on a per-roentgen basis. The incidence of dominant lethal mutations was about 2.7 percent, presumably induced mainly in post meiotic germ-cell stages, while 4.0 percent excess embryonic lethality was attributed to heritable sublethal factors, which were probably mainly heterozygous effects of recessive mutations.

The irradiated sublines did better than the control sublines with respect to some mainly maternal traits. Thus there were significantly fewer losses of whole litters in the irradiated series as far as litters 1-3 were concerned, in which litter sizes in the two series were comparable. Postnatal mortality before weaning age was reduced to almost half control values in the irradiated series, the effect showing up in both early and later litters. These findings raise the possibility that some mutations in the irradiated series were to alleles conferring postnatal heterozygous advantage on this highly inbred background, but other explanations cannot at present be excluded.

Skeletal variation with respect to 23 quasi-continuous and four metrical characters was studied in descendants of two pairs of sublines. For both types of variation the rate of divergence of the two control sublines was higher than the rate for the two irradiated sublines. The main divergence seemed to have taken place in one of the control sublines, the probable explanation being that a "major" polygenic mutation, or residual heterozygosis, had become fixed in this subline

and had thereby altered the frequencies of a number of variants simultaneously, leading to a spuriously high differentiation rate. Further work will be needed to investigate this hypothesis and derive the true rate of divergence, so that the rate of induction of polygenic mutations can be calculated.

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