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The overall rates of dominant and recessive lethal and visible mutation induced by spermatogonial x-irradiation of mice

By MARY F. LYON, RITA J. S. PHILLIPS and A. G. SEARLE

M.R.C. Radiobiological Research Unit, Harwell, Berkshire, U.K.

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1. INTRODUCTION

During the last fifteen years there has been a rapid increase in our knowledge of the relative effectiveness of different kinds, doses and intensities of ionizing radiations for the induction of mutations in the mouse. The results obtained are, however, mainly based on analysis of mutation at a set of seven specific loci, using the method described by Russell (1951). In order to gain an accurate estimate of the total mutational damage resulting from a given dose of irradiation it is necessary to supplement these specific locus data in such a way that the whole genome and all the various kinds of mutation are covered as far as possible.

Carter & Lyon (1961) attempted to measure such overall mutational damage, using a backcross method with a spermatogonial dose of 600 r. X-rays, but their results indicated that the mutation rates were lower than might have been expected and consequently that their experiment was on too small a scale to give meaningful results. Furthermore, two phenomena in the backcross generation prevented the estimation of recessive lethal incidence: (i) a significant reduction in numbers of corpora lutea in granddaughters of irradiated males as compared with controls, (ii) intra-uterine compensation. In the control series there was a significant tendency for embryonic mortality to rise with increasing numbers of implantations. This apparently led to greater mortality in the control series, in which the number of implantations was higher. This prevented the estimation of recessive lethals since the death of an embryo carrying an induced lethal mutation might increase the chances of survival of the remaining embryos by reducing intra-uterine competition, thus tending to mask induced lethal action. It seemed possible that the high embryonic loss and associated compensation were due to inbreeding depression, since the inbreeding coefficient in the backcross mothers was 50% and in the young 62·5%. Thus a new experiment was planned, and is described in this paper, in which inbreeding was avoided and in which the scale and radiation dose were increased.

The experiment was designed to provide information on the rate of induction by spermatogonial X-irradiation of the following types of mutations: (i) dominant lethals and sub-lethals acting before weaning age; (ii) dominant visibles; (iii) dominant sterility, semi-sterility and reduced fertility, due to the induction of

translocations or other factors affecting fertility; (iv) recessive autosomal lethals and sub-lethals; (v) recessive autosomal visibles.

2. METHODS

The plan of the experiment is given in Fig. 1. It was performed in three equal replicates (each carried out by one of the three authors) and was then repeated on the same scale after an interval of six months. The P_1 males were offspring of a C3H/HeH♀ × 101/H♂ inbred strain cross, as in the previous experiment. In each replicate, thirty pairs of 6–7-week-old P_1 males were collected, one of each pair

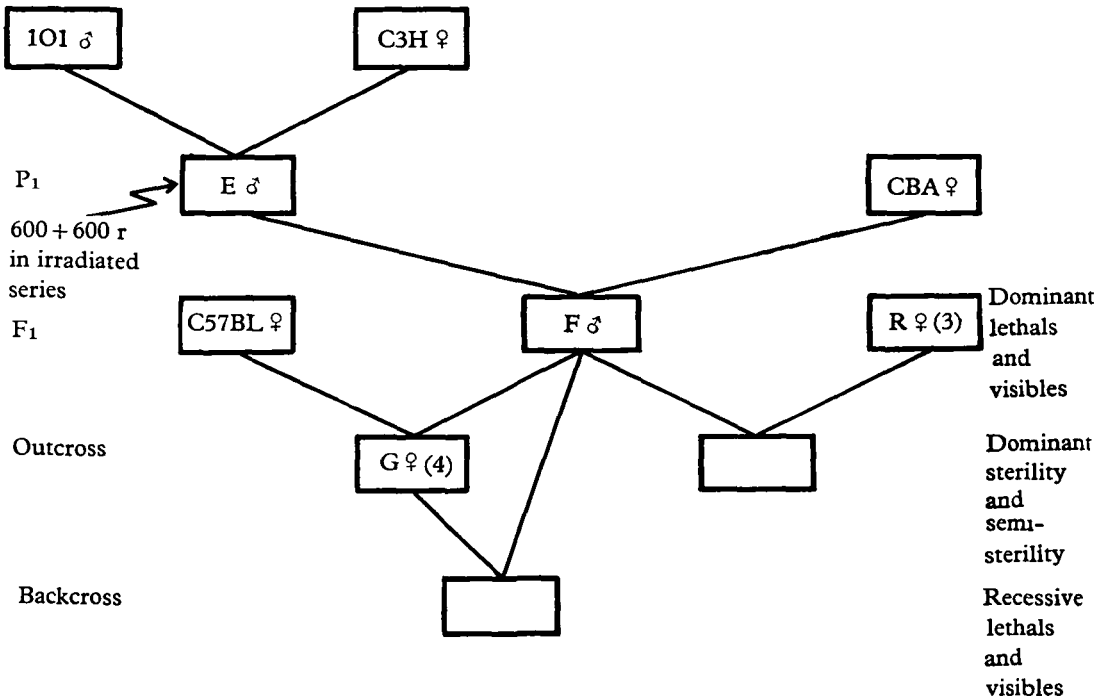

Fig. 1. Plan of experiment, showing stage of manifestation of different types of mutation.

being chosen at random for irradiation and the other used as control. A dose of 600 r. X-rays to the posterior part of the body was given at this age (250 kVp., 14 mA., HVL 1·2 mm. Cu, 217 r./min.) with another 600 r. eight weeks later. Twelve weeks after the second irradiation pairs of irradiated and control males were mated to pairs of 10-week-old CBA/H females, one brother of each pair to one sister. Each mating was allowed to produce four litters, which were sexed and examined at birth and weaning (at age $2\frac{1}{2}$ weeks). The female was killed and dissected during her fifth pregnancy at a time when her foetuses were estimated to be at about the 14-day stage, with due allowance for pregnancy prolongation in the

lactating female (Snell, 1941). Numbers of corpora lutea in each ovary and of live and dead implantations in each uterine horn were then counted. Any gross malformations in the living embryos were recorded and the dead implantations were divided into 'small moles' (due to death at or soon after implantation), 'large moles' (due to death after placentation) and dead embryos (with recognizable remains, due to death shortly before examination).

Sons from the liveborn litters were kept and tested for full fertility by mating to three outbred females, which were then dissected at 14½ days' gestation for luteal and embryonic counts. The criteria given by Carter, Lyon & Phillips (1955) were used for the recognition of semi-sterility due to translocation heterozygosity. If any of the three females gave a diagnosis of semi-sterility, or if tests with all three were inconclusive, the male concerned was not used in the rest of the experiment; instead, further tests were made to see if semi-sterility could be confirmed and if it was inherited.

In each replicate, tests were continued until fifty pairs of fully fertile males were obtained. These were then outcrossed to pairs of 10-week-old C57BL/H females and at least six daughters were kept from each male. To give the backcross generation, one daughter, picked at random, was mated to her father at 8 weeks of age. She was allowed to have three liveborn litters, which were examined at birth and weaning, and then was dissected during her next pregnancy when the foetuses were about 14 days old. Three other daughters were mated to their father when 8 weeks old, then dissected when 14½ days pregnant as before. By these procedures the presence of recessive lethal, sub-lethal and visible mutations could be detected and their time of manifestation determined.

The experiment was coded throughout, so that no unconscious bias could be introduced. Statistical analysis was by the method of paired comparisons, followed by the '*t*' test, only those pairs of matings which completed their assignment being used.

3. RESULTS

(i) *Dominant mutations*

(*a*) *Lethals*

Table 1 shows the results obtained after pooling data from all the replicates, which were on the whole in close agreement with each other. The number of liveborn young was 15·2% less in the irradiated than in the control series, while the number of offspring reaching weaning age was 15·8% less and both differences were statistically highly significant. The amount of neonatal death was very similar in the two series; and no gross congenital malformations (such as harelip and cleft palate, spina bifida or anencephaly) were found in newborn mice of either series. Little importance is attached to these observations however, since mother mice normally eat dead or dying young soon after birth.

The results from the dissections of pregnant females indicated that the main cause of these differences in numbers of offspring was a highly significant excess of death at the 'small mole' stage in the irradiated series. There was also a smaller

Table 1. *Survival and death at various ages in offspring of control and x-irradiated male mice, to show dominant lethal effects*

	Total numbers		Mean diff. (C − I)/		
	Control	Irradiated	litter	*t*	*P*
1. First four litters					
Matings	161	161			
Neonatal deaths	43	47	−0·02	—	—
Liveborn young	4537	3848	+1·07	9·91	< 0·001
Weaned young	4290	3612	+1·05	9·38	< 0·001
2. Fifth litter dissections					
Litters	155	155			
Corpora lutea*	1470	1418	+0·34	1·98	0·05
Implants	1346	1279	+0·43	2·56	< 0·02
Small moles	213	300	−0·56	3·48	< 0·001
Large moles	24	24	0·0		
Dead embryos	23	25	−0·01	–	—
Live embryos (malformed)	6	3	+0·02	4·81	< 0·001
Live embryos (normal)	1080	927	+0·99		

* Based on 152 and not 155 pairs of ovaries, owing to damage or loss preventing accurate counts on the others.

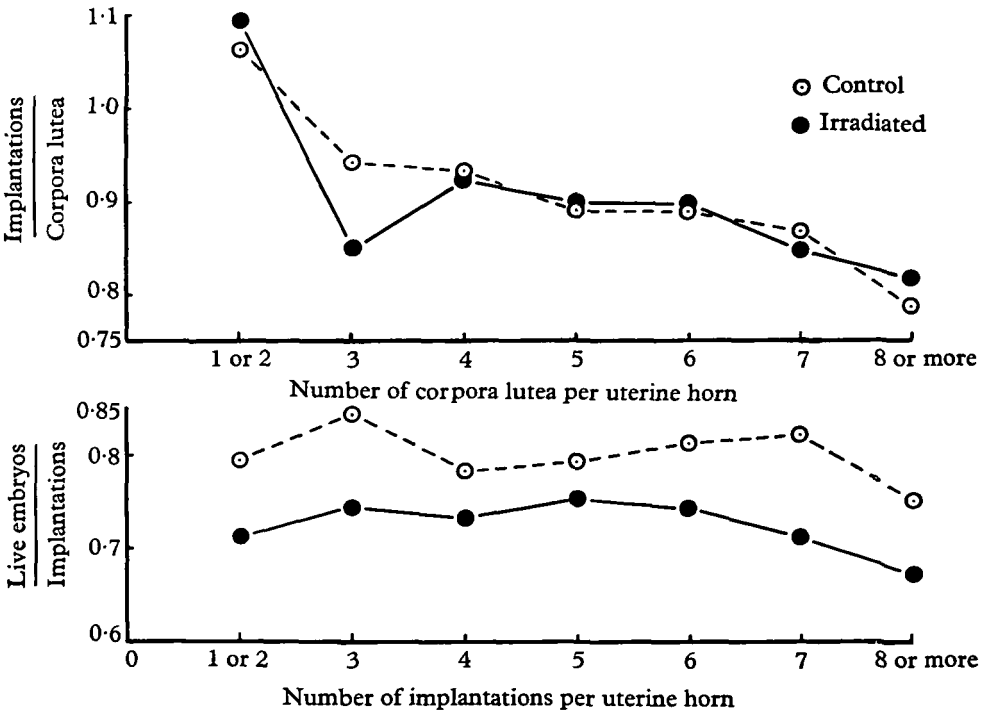

Fig. 2. Pre-implantation (above) and post-implantation (below) survival in the F_1 generation.

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difference in the numbers of implantations and of corpora lutea in the two series but no evidence of increased death in the irradiated series at stages later than the 'small mole'. The percentages of corpora lutea represented by living embryos at

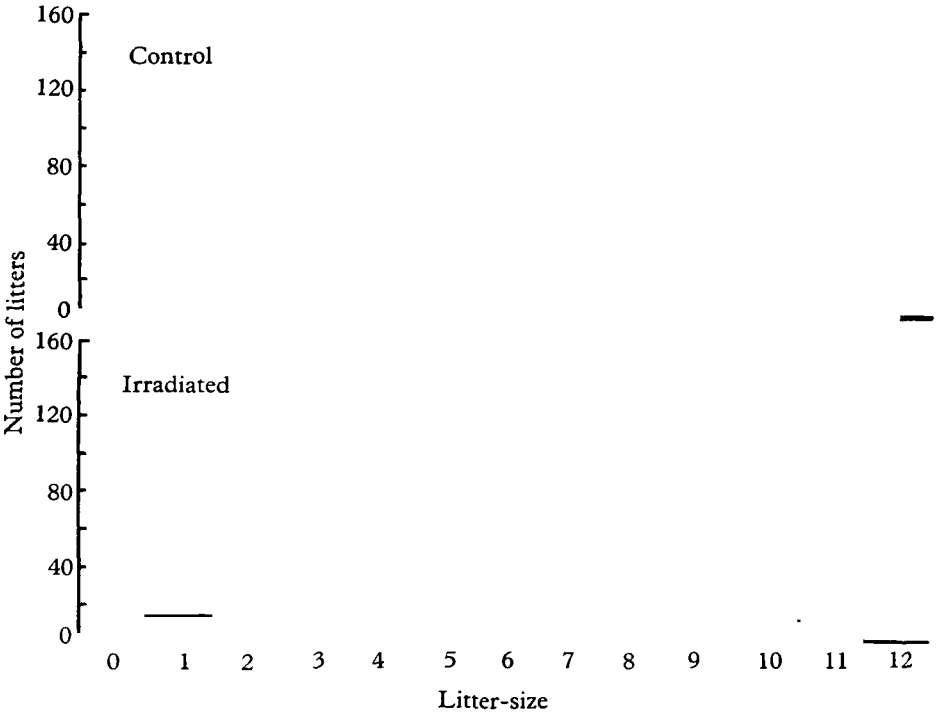

Fig. 3. Distributions of litter-sizes at birth in offspring of control and irradiated males.

14½ days were 72·3 ± 1·2 and 64·7 ± 1·3 in the control and irradiated series respectively (omitting the three pairs without full luteal counts). Therefore the rate of induction of dominant lethals calculated from the formula

$$1 - \frac{\text{living embryos/corpora lutea in irradiated series}}{\text{living embryos/corpora lutea in control series}}$$

was 0·106 or 10·6 ± 3·8%. At 14 days' gestation of the fifth litter the average number of live embryos per litter was 1·01 higher in the control series, and this agreed well with the difference of 1·07 in the average litter-size at birth in the first four litters.

There was then the question of the extent to which the effect of irradiation in causing embryonic death was being masked by a compensatory reduction in 'spontaneous' death. In Carter and Lyon's experiments the 'spontaneous' death rose with increasing numbers of implantations per uterus and since the controls had more implantations than the irradiated series they had more 'spontaneous' death. In the present crosses the difference between the series in number of im-

plantations was small. The effect of the number of embryos on mortality was investigated by plotting the pre-implantation survival rate against the number of corpora lutea, and the post-implantation survival rate against the number of implantations, each ovary and the corresponding uterine horn being counted separately (Fig. 2). In the control series, the rate of embryonic survival to implantation tended to decrease with higher luteal counts. The total number of corpora lutea was also 3·5% higher in the controls; so it is possible that there was

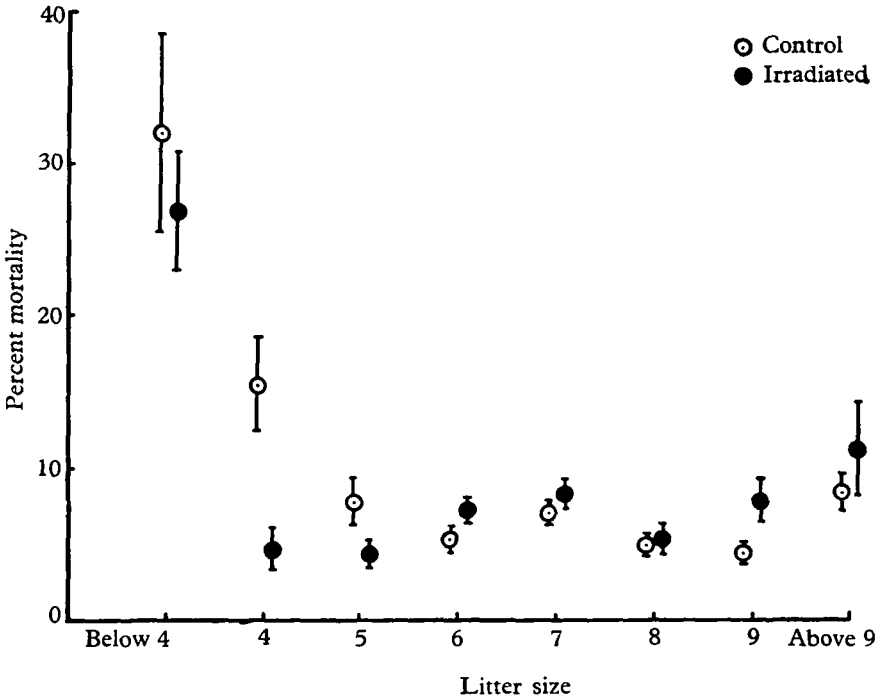

Fig. 4. Relationship between F_1 litter-size at birth and post-natal mortality up to weaning age, with standard errors.

some slight masking of induced pre-implantation lethality. In neither series, however, was there any evidence of a change in post-implantation survival rate with numbers of implantations. Thus factors tending to bias the estimation of dominant lethal mutations in these crosses seem to have had little effect, though of course the possibility of some hidden compensatory action cannot be excluded.

The observed reduction in litter-size at birth in the irradiated series resulted in there being more litters of only 1–3 young (Fig. 3). Mortality between birth and weaning in such very small litters tends to be high, probably because the stimulus for lactation is inadequate. Figure 4 shows that the mortality was indeed higher in small litters in the present crosses and hence the slight non-significant excess of mortality at this stage in the irradiated series was probably due to this effect rather than to radiation-induced dominant lethal effects.

(*b*) *Visibles*

Three heritable visible abnormalities were found in the F_1 generation, two in the irradiated series and one in the controls. These were as follows:

(i) Head spot and coat dilution Sl^H. One female in a sibship of 21 in the irradiated series had a white head-dot, lightish coat and belly, of the type found in heterozygotes for W^v (Grüneberg, 1952) or Steel, *Sl* (Sarvella & Russell, 1956). The intercrosses gave no new homozygous type, suggesting embryonic lethality. The mutant was shown not to be allelic with W^v, though the compound had a very light coat with much white, like Sl–W^v compounds. Crossed with Steel-Dickie Sl^d (Bernstein, 1960), anaemic young were produced, which died very soon after birth. Since Sl/Sl^d mice are anaemic black-eyed white, living about a week, this mutant seems to be an allele of Steel with more severe effects than Steel itself. It can be called Steel-Harwell (Sl^H).

(ii) Extra toes *Xt*. Heterozygotes for this gene show slight preaxial polydactylism and the lethal homozygotes have extreme polydactylism of all four limbs, often with cranioschisis. This mutation was found twice; in the control series of the first replicate and in the irradiated series of the second replicate. On the first occasion two out of three sons of one control male had slight protuberances on the preaxial border of both hind-feet. The abnormality was so slight that its importance was not realized until after the parents of the two original animals had been killed. It seems probable, however, that one or other of these parents was heterozygous for the gene before the experiment started and hence this particular occurrence has been disregarded. The second occurrence was in a single individual in a sibship of 17 in the second replicate. Its importance was realized from the first, and there seems no doubt it was a new mutation so this occurrence has been included.

Thus there were two dominant visible mutations in the irradiated series and none in the controls, giving an estimated rate of mutation to dominant visibles of $2/3612 = 5{\cdot}5 \times 10^{-4}$, or $4{\cdot}6 \times 10^{-7}$/gamete/r.

(*c*) *Fertility*

In all, 427 pairs of F_1 males were tested for fertility by crossing each to three outbred females, which were then dissected on the 15th day of pregnancy. As Table 2 shows, 100 of the pairs (23·4%) were not accepted for use in the second part of the experiment, because they showed some signs of infertility

Table 2. *Results of fertility tests on sons of control* (*C*) *and irradiated* (*I*) *males*

Series	No. of pairs of males tested	Accepted: fertile	Rejected: signs of infertility				With complete sterility		With heritable semi-sterility	
			C	I	Both	Total	C	I	C	I
First	208	155	18	26	9	53	0	3(1·4%)	0	8(3·8%)
Second	219	172	17	28	2	47	0	1(0·5%)	1(0·5%)	7(3·2%)
Total	427	327	35	54	11	100	0	4(0·9%)	1(0·2%)	15(3·5%)

as judged by the stringent test used. Males suspected of infertility were mated to three further females; those which seemed semi-sterile were then outcrossed to a multiple recessive stock to prove the condition heritable and for linkage tests.

Fifteen males in the irradiated series, but only one in the controls, were found to have heritable semi-sterility (Table 2). This difference is statistically highly significant, the exact probability being 0·000465. The rate of induction of semi-sterility can be estimated as 3·28%. In addition, four males in the irradiated series, but none in the controls, were completely sterile. This sterility, like the semi-sterility, may have been due to the presence of a translocation; this will be discussed later.

Meiotic stages of semi-sterile males derived from the sixteen found in this experiment were examined cytologically by the method of Welshons *et al.* (1962). All were found to be heterozygous for reciprocal translocations.

Apart from the sterile and semi-sterile animals, the numbers of control and irradiated males showing signs of infertility were very similar. This suggests that there was little induced loss of fertility in F_1 males other than semi-sterility. This belief is strengthened by the results of comparing fertilities for all those pairs in which neither male was sterile or semi-sterile, namely 407 in all (Table 3). No significant difference between the two series was found.

Table 3. *Analysis of embryonic lethality in offspring of F_1 pairs of males*, excluding *those pairs in which one member showed sterility or heritable semi-sterility*

	Control	Irradiated	Mean diff. (C − I)/male	t	P
Males tested	407	407			
Corpora lutea	11333	11422	−0·219	0·81	> 0·4
Implants	8858	8917	−0·145	0·58	> 0·5
Moles	958	1048	−0·221	1·74	> 0·05
Live embryos	7859	7829	+0·074	0·32	> 0·7

A further comparison was made using only those pairs of males accepted as fully fertile to check that there were no differences between the control and irradiated series which might be confused with recessive lethality when the males were used in backcrosses. Table 4 shows that no significant differences were present, although embryonic mortality was slightly higher in the irradiated series, with lower numbers of live embryos.

(ii) *Recessive mutations*

(*a*) *Lethals*

Evidence on the rate of induction of recessive lethals was obtained from three different sets of data. The most illuminating concerns embryonic lethality in first litters following dissections of three daughters backcrossed to each F_1 male. There are also data on litter-sizes at birth and weaning in the pairs of backcross

Table 4. *Analysis of embryonic lethality in offspring of F_1 pairs of males accepted as fully fertile*

	Control	Irradiated	Mean diff. (C − I)/male	t	P
Males tested	327	327			
Corpora lutea	9165	9128	+0·113	0·35	> 0·7
Implants	7248	7191	+0·174	0·69	> 0·4
Moles	680	731	−0·156	1·32	> 0·1
Live embryos	6537	6432	+0·321	1·20	> 0·2

matings allowed to produce three litters, with foetal data from fourth-litter autopsies of the same females.

Dealing with foetal lethality first, Table 5 shows that in both series there were significantly more moles in descendants of irradiated than of control males. There were also significantly more surviving embryos in the controls of the first series but

Table 5. *Results of dissections at* 14 *days' gestation of three females backcrossed to each F_1 male. Numbers of moles classified as large and of live embryos classified as malformed are given in brackets.*

	Series	Control	Irradiated	Mean diff. (C − I)/litter	t	P
Females dissected	I	426	426			
	II	441	441			
	Total	867	867			
Corpora lutea	I	3725	3645	+0·19	1·13	0·25
	II	3585	3690	−0·24	1·84	> 0·05
	Total	7310	7335	−0·03		
Total implants	I	3317	3205	+0·26	2·27	< 0·05
	II	3163	3266	−0·23	2·30	< 0·05
	Total	6480	6471	+0·01		
Moles	I	215(9)	284(12)	−0·16	2·64	< 0·01
	II	181(12)	303(31)	−0·28	3·52	< 0·001
	Total	396(21)	587(43)	−0·22		
Dead embryos	I	17	30	−0·03	1·83	> 0·05
	II	23	20	+0·01	0·35	> 0·7
	Total	40	50	−0·01		
Live embryos	I	3085(41)	2891(23)	+0·46	3·38	< 0·001
	II	2959(2)	2943(3)	+0·04	0·26	> 0·7
	Total	6044(43)	5834(26)	+0·24		

not of the second. This was clearly related to the surplus of corpora lutea (and therefore of pre-implantation embryos) in the controls of the first series and to the near significant deficit in those of the second. As shown below, the percentages of survival were very similar in the two series, with an excess of death in each irradiated series. This suggests the action of radiation-induced lethals rather than embryonic compensation.

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Although most of the deaths occurred at or soon after implantation, giving small moles, the excess of large moles and dead embryos in the irradiated series suggests that some of the recessive lethals were acting later, as would be expected. More live malformed embryos were found in the control than in the irradiated series, however.

There was marked heterogeneity between the six replicates with respect to numbers of corpora lutea in the control and irradiated series. In every replicate, however, the amount of post-implantation death was higher on the irradiated than on the control side.

The amount of lethality induced by the irradiation is best derived from the live embryo/corpus luteum ratios, which were as follows:

	Series I	Series II
Control	$0{\cdot}828 \pm 0{\cdot}006$	$0{\cdot}825 \pm 0{\cdot}006$
Irradiated	$0{\cdot}793 \pm 0{\cdot}007$	$0{\cdot}798 \pm 0{\cdot}007$

These ratios lead to an estimate of $4{\cdot}24 \pm 2{\cdot}37\%$ of induced lethality in the first series and $3{\cdot}37 \pm 2{\cdot}41\%$ in the second. Thus despite the lack of a difference in number of embryos alive at 14 days in series II there is no heterogeneity between the series in the percentage of mortality.

Fourth-litter dissections of the females allowed to produce three liveborn litters provide another estimate of induced foetal lethality. Results of different replicates are pooled in Table 6, as there was reasonable homogeneity. Although lethality was slightly increased in the irradiated series, and numbers of live embryos decreased, differences nowhere approached a significant level. Live embryo/corpus luteum ratios lead to an estimate of $1{\cdot}36 \pm 2{\cdot}72\%$ induced lethality.

Table 6. *Combined results of backcross fourth-litter dissections at* 14 *days' gestation*

	Control	Irradiated	Mean diff. (C − I)/litter
Litters	278	278	
Corpora lutea	2904	2908	−0·01
Total implants	2710	2698	+0·04
Small moles	200	204	−0·06
Large moles	25	24	
Dead embryos	21	26	
Live embryos (malformed)	16	1	+0·11
Live embryos (normal)	2448	2433	

Analysis of the offspring of the paired backcross matings allowed to produce liveborn young (Table 7) showed that in the first series the litter-sizes at birth and weaning were significantly lower in the descendants of irradiated mice than in controls, but in the second series were very slightly higher. Combining both series gave a non-significant reduction of 2·25% at birth and 2·54% at weaning in litter-size of the irradiated series when compared with controls. The mortality between

birth and weaning was 5·8% on the control side and 6·1% on the irradiated side, so there was little evidence for post-natal action of induced sub-lethals.

The mean differences in litter-size at birth shown in Table 7 agree fairly well with those of Table 5 relating to numbers of live embryos and suggest that few if any of the lethal mutations acted between 14½ days of foetal life and birth.

Table 7. *Numbers of young born and weaned by daughters of F_1 males*

		Pairs tested	Control	Irradiated	Mean diff. (C − I)/litter	t	P
Series							
I	Born	141	3414	3252	+0·38	2·40	<0·02
	Weaned		3279	3101	+0·42	2·41	<0·02
II	Born	145	3221	3234	−0·03		
	Weaned		2970	2989	−0·04		
Both	Born	286	6635	6486	+0·17	1·40	>0·1
	Weaned		6249	6090	+0·19	1·38	>0·1

As with the dominant lethal mutations there was then the question of any possible error in the estimation of induced lethality as a result of a compensating reduction in 'spontaneous' embryonic death. Rates of survival to implantation and to 14 days' gestation were plotted against numbers of corpora lutea and of implantations per uterine horn, respectively, using the data from the three dissected daughters of every third pair of males. Figure 5 shows that survival to implantation

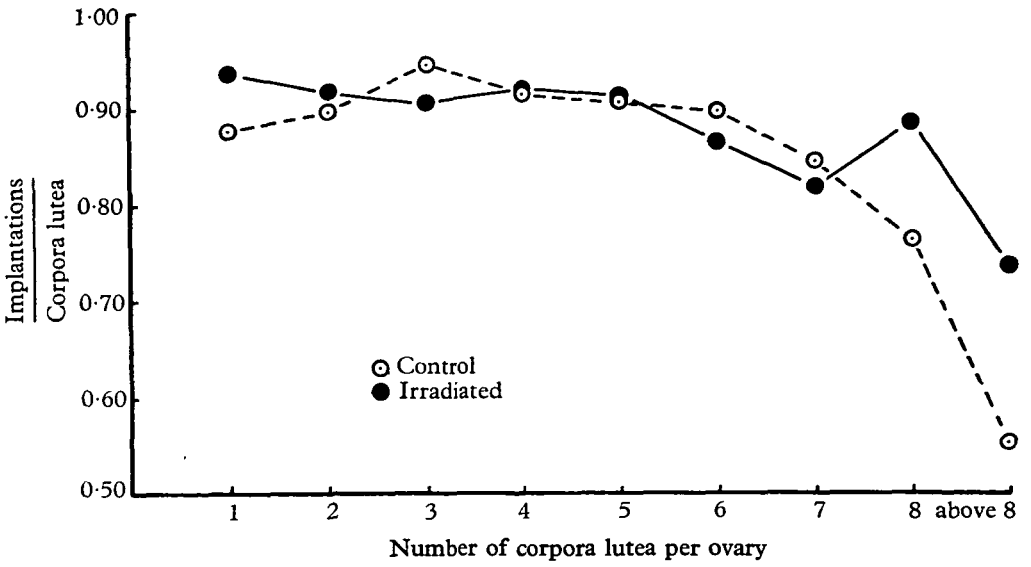

Fig. 5. Values of the implantation/corpus luteum ratio for different numbers of corpora lutea per ovary in the backcross generation.

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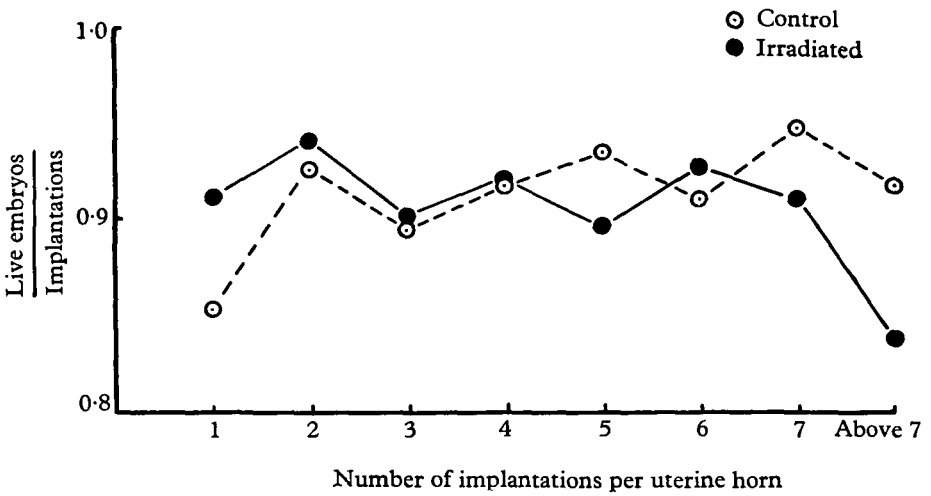

Fig. 6. Values of the live embryo/implantation ratio for different numbers of implantations per uterine horn in the backcross generation.

decreased in the controls as the number of corpora lutea rose above six, suggesting competition among embryos when these were numerous, a situation where compensation for radiation-induced lethality might occur. However, only 6% of ovaries had seven or more corpora lutea and hence, if there were any such compensation its effect could only be slight.

Figure 6 shows that the proportion of implanted embryos surviving to 14 days did not vary significantly with number of implantations in either the irradiated or

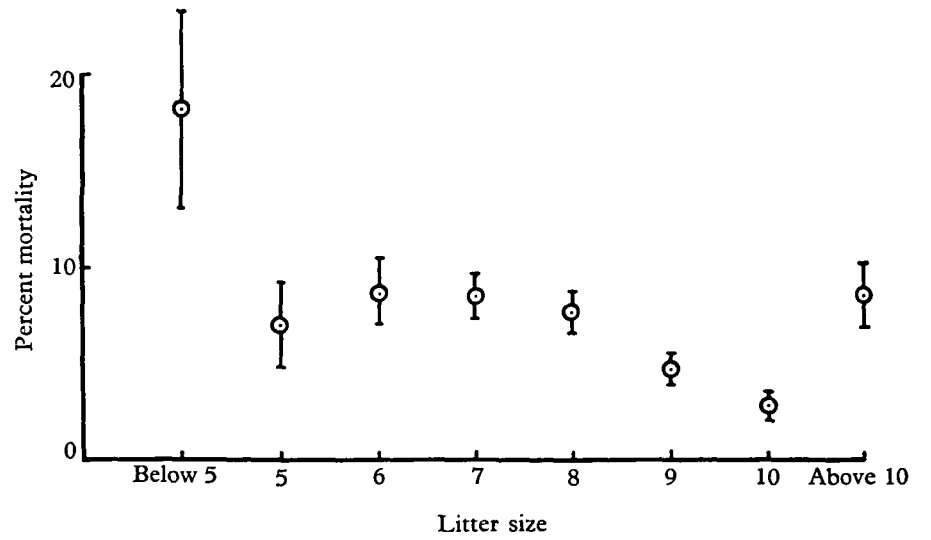

Fig. 7. Relationship between litter-size at birth in the backcross generation of the control series and post-natal mortality up to weaning age, with standard errors. Sample of results from every third mating.

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the control series and, as previously mentioned, there was no difference between series in mean number of implantations. Hence, as with the dominant lethal mutations, there is no positive evidence of any factor tending to cause error in the estimation of radiation-induced lethality.

In the control series the death rate between birth and weaning was increased in litters of less than five young (Fig. 7). As in the previous generation the irradiated series contained rather more of these small litters and hence the slightly increased death in it at this stage may not have been due to any radiation-induced lethal factors.

(*b*) *Visible mutations*

Visible mutations were looked for in the progeny of all the 286 pairs of backcross matings used in the analysis of litter-size at birth and weaning. One single-factor recessive visible was found in the control series and six in the irradiated. There was some heterogeneity with respect to these latter six, as four were found in a single replicate, and a fifth in the corresponding replicate of the other series. This may have been due to personal factors which must enter into the ability to detect mutations of an unspecified phenotype. Two of the mutations in the irradiated series were later found to be allelic with each other, and to have had a common ancestor; these have been discounted in the calculations. In addition, two visible mutations of uncertain genetic basis were found, one in each series; these also have been discounted.

The equivalent number of fully tested gametes was calculated from the distribution of progeny sizes at weaning and from Table 1 of Falconer's (1949) paper; it was 142·07 in the control and 142·02 in the irradiated series. Thus the overall mutation rate to recessive visibles can be estimated as $7{\cdot}04 \times 10^{-3}$ in the controls and $28{\cdot}17 \times 10^{-3}$ after irradiation. After correcting for control frequency, the rate of induction of recessive visible mutations is $1{\cdot}76 \times 10^{-5}$/gamete/r.

The nature of the recessive visible mutations found was as follows:

(1) *Control series*

(*a*) 'Paralysis.' The first affected animals were noticed, when 2½ weeks old, to be small and to have diarrhoea. They then developed paralysis and lack of co-ordination of all limbs and died at 3 to 3½ weeks. Similar offspring were noticed in later litters of the same parents and matings made among relatives of the affected animals suggested that the condition was due to a single recessive gene. The stock has been discarded.

(2) *Irradiated series*

(*a*) Luxoid-like. Affected homozygotes showed absence of the tibia, polydactyly of the fore-feet, and in some cases a kinky tail. Heterozygotes showed polydactyly of the hind-feet only, with incomplete penetrance. The gene concerned was found to be in linkage group II and allelic with luxoid, *lu*. There was no detectable difference in phenotype between homozygotes for

the new gene and *lulu* homozygotes. The stock has been discarded. One of the three dissected daughters of the mutant male carried the gene as well as the one which was allowed to litter.

(*b*) 'Lizard.' The first affected animals were described as having 'limited use of hind legs'. There is a partial paralysis and lack of co-ordination, particularly of the hind-limbs, leading to a 'lizard-gait'. The condition is inherited as a good recessive character, and is being investigated further.

(*c*) Abnormal embryos. One dead newborn mouse was found which was tailless, with short, malformed limbs and shortened head and jaw, and similarly malformed embryos were seen in dissections of its pregnant relatives. This abnormality again seems to be due to a single recessive gene and is being investigated further.

(*d*) Abnormal embryos. An embryo superficially similar to those just described was found during dissection of the daughter of a different F_1 male. Again the condition was due to a single recessive gene. The two genes for abnormal embryos were shown not to be allelic, and on closer inspection considerable phenotypic differences between the two types of embryos were detected. Further investigations are in progress.

(*e*) 'Circler.' The original animal was described as 'circler with slight stiffness of legs'. Subsequently, the gene concerned was found to be allelic with that for 'lizard' described above, and the irradiated males from which the two mutants were descended were brothers. From this it is concluded that the mutation which gave rise to 'lizard' and 'circler' must have occurred in an ancestor of the irradiated males, which were heterozygotes. These two mutants have therefore been discounted in calculating the results.

(*f*) Abnormal embryos. Embryos with polydactyly of all four limbs and cranioschisis were found in a dissected daughter of an F_1 male. The abnormality appeared to be due to a good recessive gene with no effect in heterozygotes. The stock has been discarded.

(3) *Conditions with uncertain genetic basis*

Two animals with white belts were found in different backcross progenies, one in each series. The white belt in the control series was shown to be inherited but to have poor fertility and the stock was lost. That in the irradiated series was inherited as a recessive with poor viability and/or penetrance. The parents and grandparents of the two mutants were unrelated. They have not been included in the calculations.

(*c*) *Sex-ratio*

The sex-ratios in the liveborn F_1 and backcross progeny are shown in Table 8. Although none of the differences reach a significant level there is a consistent tendency towards a slight increase in the proportion of males in both F_1 and backcross progeny of irradiated males.

Table 8. *Comparison of sex-ratios in descendants of control and irradiated mice, both series combined*

		BIRTH			WEANING		
		Females	Males	% Male	Females	Males	% Male
I.	*F_1 offspring*						
	Control	2227	2310	50·9	2132	2158	50·3
	Irradiated	1854	1994	51·8	1762	1850	51·2
II.	*Backcross offspring*						
	Control	3271	3361	50·7	3104	3145	50·3
	Irradiated	3161	3324	51·3	2984	3106	51·0

4. DISCUSSION

The experiment was designed in such a way as to minimize, it was hoped, those tendencies for embryonic compensation which hindered interpretation of the results obtained by Carter & Lyon (1961) and which seemed to be associated with inbreeding. This aim was largely achieved, since compensatory tendencies remained at low levels throughout the experiment. Neither did the significant deficiency of corpora lutea, found in the backcross generation of the irradiated series in Carter and Lyon's experiment, recur in the present one, where the first and second series showed non-significant differences in opposite directions. Also, the six replicates showed reasonable agreement on the whole, especially with respect to the extra lethality in the irradiated series. It seems probable, therefore, that the results represent a fairly accurate picture of the extent of mutational damage induced by the irradiation.

(i) *Dominant mutations*

It is now clear that acute x-irradiation of mouse spermatogonia results in dominant lethality, acting at about the time of implantation, and semi-sterility, due to the induction of reciprocal translocations. It is necessary to consider to what extent this dominant lethality is a secondary consequence of the induction of translocations. Each spermatogonium which is heterozygous for a translocation will be expected to produce, on the average, two spermatozoa with duplications and deficiencies besides one carrying the translocation and one with the normal chromosome complement. Zygotes carrying duplications and deficiencies tend to die around implantation, like primary dominant lethals. If, in a population of zygotes, a is the frequency of such translocation lethality, then $a/2$ is the expected frequency of translocation heterozygotes. If there is also primary dominant lethality with frequency b, then the proportion of surviving zygotes will be $1-a-b$; these will include translocation heterozygotes with a frequency of $a/2(1-a-b)$.

The frequency of radiation-induced dominant lethality in the present experiment, estimated from the live embryo/corpus luteum ratio, was 10·6%. The frequency of induced translocation heterozygosis was between 3·3% and 4·2%, depending on how many (if any) of the four sterile males in the irradiated series carried transloca-

tions; the mean (3·7%) is probably the best estimate. Thus $a+b = 0{\cdot}106$ and $a/2(1-a-b) = 0{\cdot}037$, whence $a = 0{\cdot}066$ and $b = 0{\cdot}040$.

Thus the dose of (600 + 600) r. led to the induction of translocations at an estimated rate of $3{\cdot}3 \times 10^{-2}$ per gamete (or $13{\cdot}2 \times 10^{-2}$ per spermatogonium) and of primary dominant lethal mutations at a rate of about $4{\cdot}0 \times 10^{-2}$ per gamete. This suggests that translocation induction can be an important cause of dominant lethality following spermatogonial irradiation.

About 5% of daughters of the same irradiated males used in the present experiment also showed dominant semi-sterility due to translocation heterozygosis (Searle, 1964), and Griffen (1958, 1964) too obtained semi-sterile offspring from mice in which germ-cells were irradiated at the spermatogonial stage.

L. B. Russell (1962) has discussed the extent to which F_1 male sterility following irradiation may be due to the presence of one or more translocations. It is known that X-autosome translocations are usually sterile in the male, but such a translocation could not normally be transmitted from father to son. Russell tentatively concluded that F_1 male sterility 'while very probably due to chromosome aberrations, only infrequently results from reciprocal translocations'.

If the extent of dominant lethality had been calculated from the litter-size data, it would have been nearly 50% higher than the present estimate, since there was a decrease of 15·2% in the litter-size at birth in the irradiated series as compared with the controls. There was very little evidence for any excess of late foetal or neonatal death in the irradiated series, so the main cause of the disparity seemed to be a tendency for more corpora lutea to be found in the females mated to control males than in those mated to irradiated ones.

Decreases in F_1 litter-size at weaning following spermatogonial irradiation of mice have been observed in several specific locus experiments by W. L. & L. B. Russell (1959). They obtained what they regarded as their best estimate from a 300 r. acute x-irradiation experiment, when the litter-size was reduced by about 3–4%. This agrees well with the present estimate of about four times the reduction with four times the dose, if one assumes linearity of response.

Results reported by Cox & Willham (1962) and Cox (1963) suggest that the pattern of induced dominant mortality following spermatogonial irradiation is different in swine from that in mice, with no decrease in number born, but instead a marked increase in mortality between the first and sixth days of post-natal life. They point out, however, that embryonic compensation may have masked earlier lethal effects.

The fact that only two dominant visible mutations were found in the F_1 generation is not surprising, since such mutations are recovered at very low frequency in other experiments. Phillips (1961) for instance, found only two dominant visible mutations in 10,761 mice after acute x-irradiation of spermatogonia with 600 r.

(ii) *Recessive mutations*

In this type of outcross and backcross experiment, it is important to ensure that effects of induced dominant mutations are not carried over into the backcross

generation, where all extra lethality is assumed to be recessive in origin. Analysis of fertility tests on those pairs of males diagnosed as fully fertile (Table 4) showed that there was a slight (though not significant) deficiency of live embryos in the irradiated series, amounting to 1·6%. Thus it seems possible that some dominant factors giving a slightly reduced fertility, such as inversions, escaped elimination during the fertility tests and continued into the backcross generation. But since a significant level of difference was not reached in the comparisons of Table 4, this possibility is not allowed for in the calculations which follow.

As discussed earlier, an estimate of the extent of radiation-induced recessive lethality is best derived from embryonic data. The two series of first-litter backcross dissections gave estimates of 4·24% and 3·37% induced lethality respectively whilst the pooled fourth-litter results gave an estimate of 1·36%. Taking the means of these estimates, weighted for the number of females opened in each series, we arrive at a final estimate of 3·20%. Thus survival in the irradiated series was $96{\cdot}80 \pm 1{\cdot}44\%$ of that in the controls. From this we find that the best estimate of induced recessive lethals is 29·5%, assuming that proportions of F_1 zygotes carrying 0, 1, 2 . . . induced recessive lethals follow the Poisson distribution, and that there will be an average survival rate in the backcross progeny of 7/8 with one lethal, $(7/8)^2$ with two lethals etc. Thus the rate of induction of recessive lethal mutations was about $29{\cdot}5 \times 10^{-2}$ per gamete, or $2{\cdot}46 \times 10^{-4}$/gamete/r. This is assuming that 'spontaneous' embryonic death and death due to radiation-induced lethal genes occur additively. If there were any tendency to reduction in 'spontaneous' death in litters where a lethal gene was segregating then this estimate of the mutation rate would be too low. No attempt was made to identify any individual lethals segregating in these crosses and hence it is not known whether the induced lethality found was in fact due to individual fully lethal recessive genes, or whether it was partly or wholly due to the combined effect of many minor sub-lethal genes. Hence, the measured mutation rate is to 'lethal equivalents' rather than to known lethal genes. At first sight it may seem surprising that recessive lethal mutations like dominant lethals should act mainly at the 'small mole' stage, although with slightly more spread to later stages. It should be remembered, however, that this stage covers a relatively very wide part of the embryonic development of the mouse, from the blastocyst to late somite stages, and obviously includes 'lethal periods', since most spontaneous embryonic death occurs at this stage.

In an experiment in which 276 r. acute x-irradiation was given to inbred strain CBA males for a number of successive generations, Lüning (1964) estimated that the rate of induction of recessive lethal mutations by spermatogonial irradiation was between 8 and 20×10^{-5}/genome/r. Considering the different strains, doses and methods used, there is remarkably good agreement between these two sets of results, especially since our own is likely to be an over- rather than an underestimate, as the possibility of some slight residual dominant effect cannot altogether be ruled out.

These rates are lower than might have been expected on the basis of specific locus work in the mouse. The rate of induction of specific locus recessive mutations

following 600 r. acute x-irradiation of spermatogonia is about 21×10^{-8}/locus/r. (Russell *et al.*, 1958; Phillips, 1961). Since about 75% of these mutations are lethal when homozygous, the rate of mutation to recessive lethals is about 16×10^{-8}/locus/r. If the overall mutation rate to recessive lethals is about 2×10^{-4}/gamete/r., as suggested by the estimates of Lüning and ourselves, this is only about 1250 times the specific locus rate, which implies either that the number of loci mutating in the mouse is much lower than might have been expected or that the seven loci of the specific locus experiment have mutation rates above average. Other work suggests that the mouse has at least 5000 loci (Carter, 1959), most of which should be capable of mutating to lethal alleles, whilst Lyon (1959) estimated that the number of mouse loci capable of mutation to recessive lethals probably had an upper limit of 10,000.

In *Drosophila* the results of Purdom & McSheehy (1961) and of Ytterborn (1962), on the induction of chromosome II recessive lethals following spermatogonial irradiation, would indicate overall mutation rates of about 4×10^{-5} and 5×10^{-5}/gamete/r. respectively. On the basis of specific locus work the mouse has been said to be 15 times as sensitive as *Drosophila* (Alexander, 1954; Russell, 1956) but on this showing it is only 4–5 times more sensitive. Since *Drosophila* is likely to have fewer loci than the mouse rather than more, these results again suggest that the average genetic sensitivity to radiation of mouse loci is lower than that of those used so far in specific locus experiments. Mouse and *Drosophila* results agree in that visibles are rarer than lethals in the ratio of 4–5 lethals to one visible mutation.

Turning to recessive visible mutations, the induced mutation rate in the present experiment was $1{\cdot}76 \times 10^{-5}$/gamete/r. whereas that in the specific locus experiments mentioned previously was 21×10^{-8}/locus/r., of which about one-half of the mutations were visible when homozygous in the sense used in the present experiment. Thus the present rate is less than 200 times the comparable specific locus one, and again it seems likely that the number of loci capable of mutating to recessive visibles is higher than this, and hence that the average mutation rate per locus in this experiment was below that in the specific locus experiments. There is, however, the question of personal error in detecting the unspecified mutations of the present experiment, which decreases the reliability of the results.

It has been assumed in the previous calculations that two doses of 600 r. eight weeks apart would induce twice as many mutations as a single dose. This is in line with the findings of Russell *et al.* (1959) that a fractionated dose of 1000 r. (600 r. + 400 r. fifteen weeks apart) gave a significantly higher yield of specific locus mutations than a single 1000 r. dose, the induced mutation-rate per roentgen being similar to that found at 600 r. and 300 r. The visible and recessive lethal mutations studied in the present experiment might be expected to behave in this respect like the specific locus mutations. With translocations, however, and any two-hit component of the dominant lethals, the fractionation of the dose might be expected to give a lower yield than would have been obtained from a single dose.

One of the most significant findings of the present experiment, from the point of view of genetic hazards to man, is that translocations are induced with measurable

frequency following spermatogonial irradiation. Not only do such translocations cause semi-sterility (or even full sterility on occasion) but particular ones may also lead to the production of congenital malformations, as first demonstrated by Snell *et al.* (1934) in mice and now known to occur in man also (Penrose, 1961).

Finally, these results, like those of Carter & Lyon (1961), bring out very clearly the need for a detailed study of embryonic death and survival if an undistorted picture of the overall rate of induction of lethal mutations is to be obtained.

SUMMARY

1. Hybrid (C3H♀ × 101♂) male mice were given two doses of 600 r. acute x-irradiation eight weeks apart and outcrossed at the end of their sterile period. Their fully fertile sons were outcrossed and daughters of these sons were backcrossed to them, in order to study the rates of induction in spermatogonia of dominant and recessive lethal and visible mutations, as well as dominant semi-sterility.

2. F_1 litter-size decreased by 15·2% at birth and 15·8% at weaning age, as compared with controls. This decrease was very largely due to dominant lethality acting at about the time of embryonic implantation. There was also a highly significant increase in the incidence of heritable semi-sterility, the estimated rate of induction of reciprocal translocations being 3·3% per gamete.

3. The dominant lethality was shown to be partly a secondary consequence of induced translocation heterozygosis. The estimated overall rate of dominant lethal induction was 10·6% per gamete, with 'primary' dominant lethals induced at a rate of 4·0% per gamete.

4. The estimated mutation rate to dominant visible mutations was $4{\cdot}6 \times 10^{-7}$/gamete/r., but this was based on only two mutations in the irradiated series.

5. In the backcross generation there was again significantly more embryonic death in the irradiated series, mainly at the small mole stage and this was attributed to induced lethal mutation. The survival in the irradiated series was 96·80% of that in the controls and from this the rate of induction of recessive lethals was calculated to be $2{\cdot}5 \times 10^{-4}$/gamete/r. There was no evidence of the induction of lethals acting later than 14 days' gestation.

6. The estimated rate of induction of recessive visible mutations was $1{\cdot}8 \times 10^{-5}$/gamete/r., but the results showed heterogeneity, probably due to personal factors.

7. No significant sex-ratio differences were found.

8. The results were compared with those of specific locus and other relevant experiments. The rates of induction of recessive lethal mutations and of recessive visibles were both lower than might have been expected. On these results the mouse was only 4–5 times more sensitive than *Drosophila* to the mutagenic effects of radiation.

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