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An attempt to estimate the induction by X-rays of recessive lethal and visible mutations in mice

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INTRODUCTION

The most efficient technique now available for the study of mutation rates in the mouse, *Mus musculus* L., is the 'specific-locus method' of looking for mutations to recessive alleles at a small number of specified loci. This method has been very valuable for comparing the mutagenic effects of different radiation doses (Russell, Russell & Kelly, 1958; Russell, Russell & Cupp, 1959), and the sensitivities of various germ-cell types and stages (Carter, 1958; Carter, Lyon & Phillips, 1960; Russell, Bangham & Gower, 1958), but it has the inherent limitation of giving no information about mutation at mouse loci in general. The experiment described in the present paper was devised to supplement the specific-locus work by measuring the mutation rates of whole classes of genes, using a radiation dose and a mouse stock for which much specific-locus information was already available. It was thus intended to form a bridge between the specific-locus work and work on the more general genetic effects of radiation on fitness, quantitative characters and so on.

Previously an attempt had been made to measure the mutation rate to recessive lethal genes linked to known visible genes, in the hope that this would prove a useful technique for studying mutation to recessive lethals (Carter, 1959). This experiment gave unexpectedly negative results, however, suggesting that the effective number of loci mutating to recessive lethals might be rather low. Accordingly, the present experiment was designed to obtain as much information as possible from the animals to be used, including any incidental results concerning dominant mutations. In all, it was hoped to obtain information about dominant visibles, lethals and semisteriles and about recessive visibles and lethals.

The method was basically the 'backcross method' of detecting recessive mutations. Treated animals (P_1 generation) are mated to unrelated normal animals, giving an F_1 generation expected to exhibit dominant mutations induced in the parent and to carry recessive ones heterozygously. F_1 males are then outcrossed to unrelated normal animals and their daughters backcrossed to them. One-half of the daughters will carry the recessive mutants present in their sires, so that these mutants will segregate in the backcross generation. Recessive visible mutants are

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detected by inspection of liveborn young; recessive lethals, in the present experiment, were detected by comparisons of litter-sizes and of numbers of live and dead embryos in the uteri of females dissected when pregnant. A detailed plan of the experiment follows (Fig. 1).

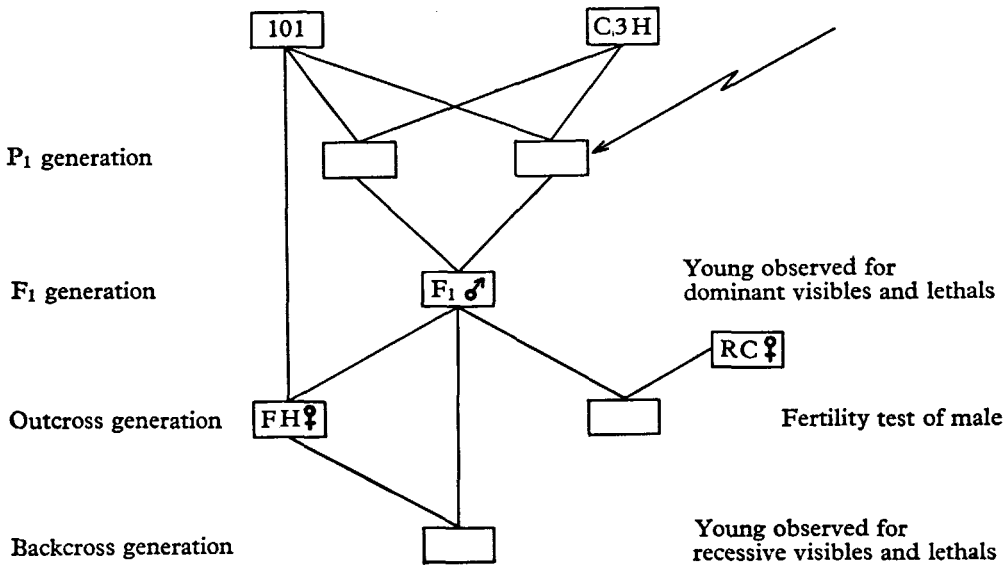

Fig. 1. Plan of the breeding scheme used in the experiment.

METHODS

(i) *Irradiation*

The P_1 animals irradiated were crossbred males from the line cross C3H/HeH × 101/H. Twenty-eight of these males were given 600 r X-rays (250 Kvp, 14 ma, HVL 1·2 mm Al; 67·6 r/min.) to the whole body when 8 weeks old. This stock and radiation dose were chosen since Russell, Russell & Kelly had bred many thousands of mice in specific-locus experiments using a similar stock and dose. For each irradiated male a litter-brother was kept as a control.

(ii) *Breeding*

A previous experiment had shown that male mice irradiated in this way recovered their fertility 13 weeks after irradiation. Therefore in this experiment males were mated 6 weeks later than this, i.e. 19 weeks after irradiation. The twenty-eight pairs of irradiated and control P_1 males were mated to pairs of litter-sisters from the same type of cross, one brother of each pair to one sister of each pair. Each female was allowed to produce four liveborn litters, which were observed for dominant visible mutations. To detect dominant lethal mutations she was killed in her fifth pregnancy at about 14 days' gestation and dissected. The numbers of corpora lutea in her ovaries, and of live and dead embryos in her uterus were counted.

From the liveborn litters fifty-six pairs of sons (F_1 generation) were kept, each son from an irradiated male being paired with a son from the corresponding control male. To detect any complete or partial reduction of fertility of these F_1 males they were mated to three cross-bred females each; the females were dissected when 14 days pregnant and their embryos and corpora lutea counted. Any F_1 male found to be partially or wholly sterile was then discarded, together with his pair.

After the fertility test the pairs of F_1 males were mated to pairs of litter-sisters of strain 101/H, to provide daughters for the backcross matings. These females were allowed to produce four liveborn litters and were dissected when again pregnant (thus providing a further check on male fertility).

In order to detect the action of recessive lethal genes three daughters of each F_1 male were backcrossed to their sire and dissected when pregnant. Two other daughters were backcrossed and allowed to raise seven litters each, which were examined for recessive visible mutants and for litter-size at birth and weaning.

Statistical tests on the results were made by paired comparisons, taking each male from the irradiated series and his corresponding control as a pair. Hence, when it was necessary to discard any male who had failed to complete his schedule, his opposite number in the other series was also discarded.

Throughout all the breeding procedures the individuals of each pair of P_1 and F_1 males were known by code letters which gave no indication which of the pair was from the irradiated series and which the control. This was in order to avoid any unconscious bias by those handling them.

RESULTS

1. *Results of matings of the irradiated and control males*

(*a*) *Dominant visibles*

No dominant visible mutations were observed among 854 weaned offspring of control males and 838 offspring of irradiated males. This is in accord with previous results; Russell (1951) after giving 600 r X-rays to male mice found only 5 dominant visible mutations in 30,000 offspring.

(*b*) *Dominant lethals*

Table 1 shows, for those pairs of P_1 males which completed the schedule, the numbers of liveborn young in four litters, and the results of dissecting the females during their fifth pregnancy. Eight of the original twenty-eight pairs of P_1 males were discarded, in five cases because one or both P_1 females developed a tumour and in the other three cases because one or both P_1 males were sterile.

There were no significant differences between the series, whether in numbers of implants, of live foetuses or of liveborn young. Thus any dominant lethals present in the gametes of the irradiated P_1 males were too few to be detected on the scale of the present experiment. The observed difference in number of live foetuses per female was $0{\cdot}30 \pm 0{\cdot}52$, which corresponds to an incidence of $3{\cdot}2\%$ dominant lethals, with an upper fiducial limit of $14{\cdot}3\%$. Similarly, the observed difference

in number of liveborn young corresponds to an incidence of 2·7 % dominant lethals, and an upper fiducial limit of 8·8 %.

Table 1. *The results of a search for dominant lethals in the germ-cells of the irradiated males*

	Control	Irradiated	Mean difference and S.E.	t	P
No. of males	20	20			
Liveborn young (4 litters per male)	731	711	$+1{\cdot}00 \pm 1{\cdot}11$	0·91	> 0·3
Dissections in fifth pregnancy					
Corpora lutea	216	220	$-0{\cdot}20 \pm 0{\cdot}50$	−0·40	> 0·6
Implants	209	211	$-0{\cdot}10 \pm 0{\cdot}43$	−0·23	> 0·8
Live embryos	187	181	$+0{\cdot}30 \pm 0{\cdot}52$	+0·58	> 0·5
Moles and dead embryos	22	30	$-0{\cdot}40 \pm 0{\cdot}37$	−1·07	> 0·2

No significant differences between control and irradiated series in any character.

This again is in line with previous results. Various workers have reported that, though many dominant lethals are detectable among offspring sired in the first few weeks after irradiation, very few, if any, are found among offspring sired after the sterile period. Russell (1954) found a 3 % decrease in litter-size at weaning in the post-sterile period after giving 600 r to males; the present data are consistent with such an effect but too few to measure it.

2. *Fertility tests of F_1 males*

Fifty-six F_1 males were tested in each series. One control male was fully sterile; no male in either series proved to be semisterile according to the criteria used by Carter, Lyon & Phillips (1955) for translocation heterozygotes.

In addition Table 2 shows that there were no detectable differences between the irradiated and control series in number of implants per uterus, nor in the number of implants having live embryos, i.e. there was no evidence of the induction of mutations to dominant genes reducing male fertility.

After these fertility tests the F_1 males were mated to females of strain 101/H which were allowed to produce four liveborn litters each. There was no significant difference between the series in litter-size at birth, and thus again no evidence of dominant reduction of fertility.

Table 2. *Results of fertility tests of F_1 males*

	Control	Irradiated	Mean difference and S.E.	t	P
No. of females opened	140	140			
Corpora lutea	1381	1376	$+0{\cdot}036 \pm 0{\cdot}268$	+0·133	> 0·89
Implants	1138	1131	$+0{\cdot}050 \pm 0{\cdot}270$	+0·185	> 0·85
Live embryos	1019	1003	$+0{\cdot}114 \pm 0{\cdot}279$	+0·409	> 0·68
Moles and dead embryos	119	128	$-0{\cdot}064 \pm 0{\cdot}121$	−0·534	> 0·59

No significant differences between the series in any character.

3. *Backcross matings of the F_1 males*

(*a*) *Recessive visibles*

The liveborn backcross young were examined at weaning age for the segregation of recessive mutant genes with a visible effect. None was found in either series.

In order to attempt to set an upper fiducial limit to the mutation rate Falconer's (1949) method was used to estimate the equivalent number of fully tested gametes. This method takes into account the fact that, in an experiment of this type, the probability of detecting a recessive mutant carried by an animal under test will always be less than unity. This is because each animal can only be tested by a small number of daughters, and a limited number of young from each daughter. The efficiency of test, or probability of detecting a mutant if present, is found for each F_1 male, and the equivalent number of fully tested gametes is obtained by summing the efficiencies of test of all the males.

Table 3 shows that in the control series of the present experiment the equivalent number of fully tested gametes was 34·2 and in the irradiated series 34·0. Taking

Table 3. *Equivalent numbers of gametes tested for recessive visible genes*

	Control		Irradiated	
	No.	Equiv. gametes	No.	Equiv. gametes
Males with young reared by two daughters	44	32·6	45	33·5
Males with young reared by one daughter	4	1·6	1	0·5
Males with no young reared	8	0	10	0
Total	56	34·2	56	34·0

the upper fiducial limit of the observed number of mutations as 3·69 at the 5% probability level (Fisher & Yates, 1953, Table VIII *i*) gives an upper limit to the mutation rate in the irradiated series of 3·69/34·0 or 0·1085. Since the radiation dose was 600 r this is equivalent to an upper limit of 0·000181 recessive visibles per haploid set per roentgen.

(*b*) *Recessive lethals*

In order to detect the action of possible recessive lethals at various stages of embryonic and foetal life of the mouse it is necessary to study separately the rates of embryonic loss at these various stages. Table 4 shows the results obtained from the dissections of pregnant females and observations of litter-size at birth. Although females were allowed to produce up to seven litters, only first litters are included in this table. This is to make the observations on liveborn litters comparable with those on the dissections, which were all first pregnancies.

The number of implantations per uterus in dissected females was lower in the irradiated series than in the controls, and the difference was statistically significant. This suggests the induction in the irradiated series of recessive lethal genes causing death of embryos before implantation. However, there was also an (unexpected) significant difference in number of corpora lutea which could account

Table 4. *Results of backcross matings; dissections and liveborn first litters*

	Control	Irradiated	Mean difference and S.E.	t	P
No. of females dissected	132	132			
Corpora lutea	1423	1321	$+0{\cdot}773 \pm 0{\cdot}335$	2·309	$< 0{\cdot}03$
Implantations	1152	1076	$+0{\cdot}576 \pm 0{\cdot}199$	2·895	$< 0{\cdot}01$
Live implants	1039	995	$+0{\cdot}333 \pm 0{\cdot}225$	1·483	$> 0{\cdot}1$
Moles	96	58	$+0{\cdot}288 \pm 0{\cdot}116$	2·491	$< 0{\cdot}02$
Dead and malformed	17	23	$-0{\cdot}045$	—	—
No. of first litters	84	84			
No. liveborn young	600	635	$-0{\cdot}416 \pm 0{\cdot}290$	1·439	$> 0{\cdot}1$

entirely for the difference in number of implants, the percentage implantation in the two series being almost exactly equal. Thus there is no evidence for the induction of lethals acting before implantation, and lethals acting later must be detected by an increase in the difference between series already present at implantation. However, when the numbers of embryos alive at $14\frac{1}{2}$ days are compared the difference between irradiated and controls is smaller, and not statistically significant. Moreover, in number of young alive at birth in first litters the irradiated series did better than the controls, although again the difference is not statistically significant.

This reversal of the difference between irradiated and control series as development proceeded suggested that compensation was occurring. It seemed possible that where there were more implants in a uterus there might be more foetal death, and where more foetuses were born there might be more neonatal death. If this were so, it might be possible to detect such compensation within as well as between series. A test within the control series (Table 5) showed that the proportion of moles and dead embryos increased as the total numbers of implants per uterus

Table 5. *Relation of proportion of dead implants to number of implantations per uterus in the control series*

No. of implantations	No. of uteri	Moles and dead embryos	Live embryos	% dead
3	1	0	3	
4	1	0	4	0
5	2	0	10	
6	11	3	63	4·5
7	16	6	106	5·4
8	24	16	176	8·3
9	29	28	233	10·7
10	30	19	281	6·3
11	14	31	123	20·1
12	3	5	31	
13	—	—	—	20·0
14	1	5	9	

$\chi^2_{[2]} = 19{\cdot}76. \quad P < 0{\cdot}001.$

χ^2 was calculated by a method due to Holt (1948): Let n_1, n_2 be the total numbers of live embryos and of moles and dead embryos, and let a be the number of implantations.

Then

$$\chi^2 = \frac{(n_2 S(a_1) - n_1 S(a_2))^2}{n_1 n_2 S(a - \bar{a})^2}$$

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increased. Thus, since the mean number of implants per uterus was higher in the control series, this effect might be sufficient to account for its higher amount of embryonic loss.

Table 6. *Numbers of liveborn offspring of backcross matings*

Litter order	No. of litters		No. liveborn		Liveborn per litter	
	C	I	C	I	C	I
1	92	91	641	672	6·97	7·38
2	85	86	589	624	6·93	7·26
3	77	78	523	521	6·79	6·68
4	67	74	449	496	6·70	6·70
5	64	67	405	410	6·33	6·12
6	56	60	309	353	5·52	5·88
7	50	56	269	297	5·38	5·30
Totals	491	512	3185	3373	6·487	6·588
					I/C = 1·0156	

C = Control; I = Irradiated.

Unfortunately, the amount of compensation occurring cannot be measured and there is no means of knowing whether or to what extent recessive lethal genes were also acting. Thus no estimate of the mutation rate to recessive lethals can be made from these data. However, although one cannot hope to measure a mutation rate, it is still of interest to compare the numbers of progeny in the control and irradiated series, to obtain a general idea of the overall effect of the irradiation on fertility. Tables 6 and 7 give more information about the numbers of liveborn and weaned young in the backcross matings. The data are remarkable for the absence of effect of the radiation. It has already been noted that in first litters the number of liveborn young was slightly higher in the irradiated series than in the controls. This held true even when the whole seven litters were considered, though the difference was small, whereas in number of young weaned, and percentage weaned of young born, the irradiated series was slightly worse than the control. In no cases were the differences statistically significant.

Table 7. *Numbers of weaned offspring of backcross matings*

Litter order	No. of litters		No. weaned		Weaned per litter	
	C	I	C	I	C	I
1	92	91	588	557	6·39	6·12
2	85	86	557	573	6·55	6·66
3	77	78	498	489	6·47	6·27
4	67	74	409	480	6·10	6·49
5	64	67	388	384	6·47	5·73
6	56	60	289	333	5·16	5·55
7	50	56	236	281	4·72	5·02
Totals	491	512	2965	3097	6·076	6·061
					I/C = 0·9975	

	% weaned
Control	93·09 ± 0·45
Irradiated	91·82 ± 0·47

DISCUSSION

The main interest of these results lies in the relative absence of effect of the radiation.

The experiment was designed primarily to measure overall mutation rates to recessive visible and recessive lethal genes in the mouse, but no recessive visible mutations were observed and the action of recessive lethals could not be detected. However, evidence from other experiments indicates that mutations to both these classes are in fact induced by radiation in the mouse. In Russell, Russell & Kelly's (1958) specific-locus experiments at the same radiation dose the average mutation rate per locus was 20×10^{-8}/r. Of these mutants some 20% were viable when homozygous (Russell & Russell, 1959) and could have been detected as recessive visibles in the present experiment; the remainder could have been detected as recessive lethals. The estimated upper limit to the mutation rate to recessive visibles in the present experiment was $18{\cdot}1 \times 10^{-5}$/r; this is 900 times the specific locus rate, or 4500 times the rate to viable alleles only. It is hoped that a larger-scale experiment, now in progress, will give an actual measure of the mutation rate to recessive visibles.

From the attempt to measure recessive lethals two main points emerged. The first was the significant difference in corpus luteum count between the females of the control and irradiated series. This suggests some general deleterious genetic effect of the radiation, dominant in effect (since the females were the progeny of an outcross), and probably polygenic. The second interesting fact was that this difference in number of corpora lutea was not reflected in any difference in litter-size at birth or weaning, and that, in general, the radiation appeared to have had little effect on these characters. Apparently, in any female, embryonic mortality was inversely related to the number of embryos present and this tended to compensate for differences in numbers of corpora lutea and implantations. Somewhat similar effects were found by Bowman & Roberts (1958), who reported that in any uterine horn of the mouse the loss of ova between shedding and implantation increased as the number of eggs shed by the corresponding ovary increased. Edwards & Fowler (1959) and McLaren & Michie (1959), both studying females in which the number of eggs shed had been artificially increased by superovulation, found that death in the middle and later periods of pregnancy increased when the number of implants in a uterine horn rose, and that the number of young born alive was inversely related to the number of implants. Neither of these findings is exactly comparable with that in the control series of the present experiment, which was that the proportion of moles and dead embryos increased with the number of implants. However, since it is well known that the amount of embryonic loss in mice varies greatly both from stock to stock and through environmental causes, it seems scarcely surprising that compensation, if it occurs, should be detectable at different stages in different stocks.

It is possible that some of the embryonic loss in the present experiment was attributable to inbreeding depression. Roberts (1960) and Bowman and Falconer (1960) found that, on the average, litter-size at birth decreased by about 0·5 mice

for each 10% increase in the inbreeding coefficient. The reduction was due to reduced implantation rather than to a decreased ovulation rate (Falconer & Roberts, 1960). The animals of the backcross generation of the present experiment were quite highly inbred; the inbreeding coefficient for the mothers was 50%, and for the young 62·5%. Therefore, the inbreeding could have caused a reduction in litter-size of as much as 3·0 mice per litter. This is potentially large in comparison with the effect of the radiation given, since the induction of one recessive lethal per sperm in the irradiated series would only reduce the litter-size to seven-eighths of the control value. The inbreeding of course acts equally on irradiated and control series, and should therefore not affect the detection of induced lethals, *provided* that deaths due to the two causes occur independently. If there is compensation, however, the inbreeding effect is potentially more than large enough to mask the radiation effect. In the new experiment mentioned above inbreeding is being kept to a minimum. Lüning (1960) is conducting a somewhat similar experiment within an inbred line, CBA. He has found no statistically significant deleterious effect of radiation so far, in the first generation of an experiment that is to cover several generations.

Thus there are two main points brought out by the present experiment. The first is that theoretical extrapolations from results of experiments involving a few gene loci to the effect on the genome as a whole are not justified. Although it is known that 600 r X-rays delivered to a male mouse induces recessive lethal mutations, the effect in this experiment was that the litter-size in the irradiated series was no lower than in the control group. The second point concerns the danger in arguing from one species to another. The absence of effect here may have been due, at least in part, to intra-uterine compensation. In a monotocous species such compensation could not occur, and the results might be very different.

SUMMARY

The experiment was designed to form a bridge between the results of specific-locus experiments, using only a few gene loci, and those using the whole genome of the mouse. Male mice were given 600 r acute X-rays and bred from in such a way that at successive stages mutation in spermatogonia to dominant visibles and lethals, dominant semisteriles, recessive visibles and recessive lethals could be measured. The data concerning dominant mutations were relatively few but confirmed previous results. No recessive visible mutations were found, and the upper fiducial limit to the induced mutation rate to recessive visibles was set at a value 4500 times the rate to viable specific-locus mutations. From the attempt to measure recessive lethal mutations two interesting points emerged. The first was that granddaughters of the irradiated males had fewer corpora lutea per pregnancy than granddaughters of the control males, and the second was that this difference in number of ova shed was not reflected in any difference in litter-size at birth. Since this suggests intra-uterine compensation, no attempt was made to calculate mutation rates to recessive lethal genes from these data. The implications of the results are discussed.

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REFERENCES

BOWMAN, J. C. & FALCONER, D. S. (1960). Inbreeding depression and heterosis of litter-size in mice. *Genet. Res.* **1**, 262–274.

BOWMAN, J. C. & ROBERTS, R. C. (1958). Embryonic mortality in relation to ovulation rate in the house mouse. *J. exp. Biol.* **35**, 138–143.

CARTER, T. C. (1958). Radiation-induced gene mutation in adult female and foetal male mice. *Brit. J. Radiol.* **31**, 407–411.

CARTER, T. C. (1959). A pilot experiment with mice, using Haldane's method for detecting induced autosomal recessive lethal genes. *J. Genet.* **56**, 353–362.

CARTER, T. C., LYON, M. F. & PHILLIPS, R. J. S. (1955). Gene-tagged chromosome translocations in eleven stocks of mice. *J. Genet.* **53**, 154–166.

CARTER, T. C., LYON, M. F. & PHILLIPS, R. J. S. (1960). Genetic sensitivity to X-rays of mouse foetal gonads. *Genet. Res.* **1**, 351–355.

EDWARDS, R. G. & FOWLER, R. E. (1959). Fetal mortality in adult mice after superovulation with gonadotrophins. *J. exp. Zool.* **141**, 299–322.

FALCONER, D. S. (1949). The estimation of mutation rates from incompletely tested gametes, and the detection of mutations in mammals. *J. Genet.* **49**, 226–234.

FALCONER, D. S. & ROBERTS, R. C. (1960). Effect of inbreeding on ovulation rate and foetal mortality in mice. *Genet. Res.* **1**, 422–430.

FISHER, R. A. & YATES, F. (1953). *Statistical Tables for Biological, Agricultural and Medical Research.* Edinburgh: Oliver and Boyd.

HOLT, S. B. (1948). The effect of maternal age on the manifestation of a polydactyl gene in mice. *Ann. Eugen., Lond.*, **14**, 144–157.

LÜNING, K. G. (1960). Studies of irradiated mouse populations. 1. Plans and report of the first generation. *Hereditas*, **46**, 668–674.

MCLAREN, A. & MICHIE, D. (1959). Superpregnancy in the mouse. 1. Implantation and foetal mortality after induced superovulation in females of various ages. *J. exp. Biol.* **36**, 281–300.

ROBERTS, R. C. (1960). The effects on litter-size of crossing lines of inbred mice without selection. *Genet. Res.* **1**, 239–252.

RUSSELL, W. L. (1951). X-ray induced mutations in mice. *Cold Spr. Harb. Sym. quant. Biol.* **16**, 327–336.

RUSSELL, W. L. (1954). Genetic effects of radiation in mammals. *Radiation Biology*, ed. A. Hollaender, Vol. 1, Ch. 12, pp. 825–859. New York: McGraw-Hill.

RUSSELL, W. L., BANGHAM, J. W. & GOWER, J. S. (1958). Comparison between mutations induced in spermatogonial and postspermatogonial stages in the mouse. *Proc. Xth int. Cong. Genet.* **2**, 245–246.

RUSSELL, W. L. & RUSSELL, L. B. (1959). The genetic and phenotypic characteristics of radiation-induced mutations in mice. *Rad. Res.* suppl. 1, 296–305.

RUSSELL, W. L., RUSSELL, L. B. & KELLY, E. M. (1958). Radiation dose rate and mutation frequency. *Science*, **128**, 1546–1550.

RUSSELL, W. L., RUSSELL, L. B. & CUPP, M. B. (1959). Dependence of mutation frequency on radiation dose rate in female mice. *Proc. nat. Acad. Sci., Wash.*, **45**, 18–23.