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The effect of dose-rate on the yield of translocations and dominant lethals following spermatogonial irradiation of mice

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1. INTRODUCTION

The dose-rate phenomenon with respect to the induction of specific locus mutations in mice, following treatment of immature germ-cells with low LET radiation, has been investigated in detail by W. L. Russell and associates (cf. Russell, 1963) with confirmatory and other studies by one of us (Phillips, 1961). Quantitative data are now becoming available on the rate of induction of translocations and dominant lethals following spermatogonial x-irradiation (Lyon *et al.*, 1964; Searle, 1964). It is therefore possible to find out to what extent there is a similar dose-rate effect with chromosomal mutations, leading to a lower yield when irradiation is given at low intensity over a prolonged period. The present paper shows that such an effect does exist, though its mechanism may be quite different from that responsible for the dose-rate effect with point mutations.

2. METHODS

The plan of the experiment (Fig. 1) follows as closely as possible that of Lyon *et al.*, using the same stocks and the same combination of liveborn and foetal examination. Six-week-old male hybrids between the highly inbred strains C3H/HeH and 101/H were irradiated nightly with ^{60}Co γ-rays for twelve weeks, accumulating 100 r./week and 1200 r. in all, the instantaneous dose-rate being about 0·017 r./min. The males became sterile, but later regained fertility. Twelve weeks after the end of the irradiation they were mated to 8–10-week-old strain CBA/H females; at the same time unirradiated brothers of the males were mated to sisters of the females, to act as controls. The experiment was coded to avoid bias. It was carried out in two simultaneously replicates, each under the charge of one author.

The mated pairs were allowed to have four litters, which were sexed and examined at birth and weaning. The female was killed and dissected during her fifth pregnancy, when live foetuses were judged to be at about the 14-day stage of development. Numbers of corpora lutea, live and dead implants were counted. 216 sons in each series were mated to three outbred 'R' females, to test for semi-sterility by the criteria of Carter *et al.* (1955), at least one female having to give a diagnosis of semi-sterility for further tests to be made. If all three tests were inconclusive,

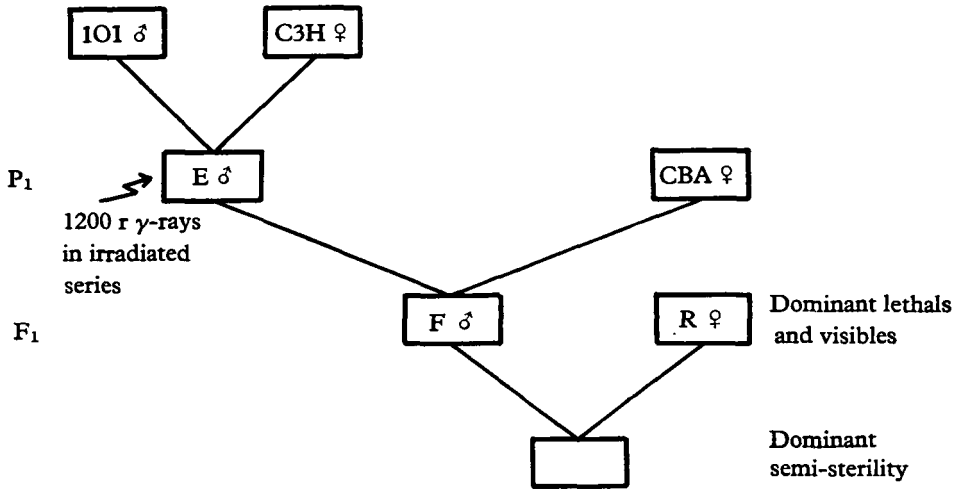

Fig. 1. Plan of the experiment, showing stage of manifestation of different types of mutation.

however, then another three females were crossed to the male concerned. Inherited semi-sterility was confirmed by showing the presence of semi-sterile progeny, with cytological confirmation of translocation heterozygosity, using the method of Welshons *et al.* (1962).

3. RESULTS

Data from dissections and from litter-size at birth and weaning (Table 1) gave very little evidence for the induction of dominant lethals or sub-lethals by the spermatogonial irradiation. Survival to 14 days of embryonic life in the irradiated series was 98·2% that in the controls, as measured by the live embryo/corpus luteum ratio, but this and other differences did not reach a significant level. Comparison of the percentages of implantation with different numbers of corpora

Table 1. *Survival and death in offspring of* 1200 *r. γ-irradiated male mice, compared with controls*

	Control totals	Irradiated totals	Mean difference (C − I) per litter	t	P
1. First four litters:					
Mated pairs	157	157			
Born	4001	4017	−0·04	0·16	> 0·9
Weaned	3519	3510	+0·03	0·07	> 0·9
2. Fifth litters *in utero*:					
Litters examined	143	143			
Corpora lutea	1296	1303	−0·05	0·25	> 0·8
Implants	1107	1094	+0·12	0·45	> 0·6
Moles	218	220	−0·09	0·08	> 0·9
Live embryos	863	852	+0·13	0·33	> 0·7

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lutea and the percentages of embryonic survival to 14 days with different numbers of implantations showed that compensatory tendencies (Carter & Lyon, 1961) were negligible in this experiment, as in the experiment of Lyon *et al.* (1964), with the same stocks. Thus there is no evidence for the induction of lethal factors whose action is masked by intra-uterine selection. There were no confirmed examples of dominant visible mutation. Four mice with abnormal phenotypes were recorded in each series, but either they died before testing or the condition was shown not to be inherited.

Fertility tests on F_1 males gave the following results:

Series	Fertile	Semi-sterile	% Semi-sterile
Control	216	0	0·00
Irradiated	214	2	0·93

The difference is not significant.

Data from 212 pairs of F_1 males were suitable for a detailed comparison of embryonic death and survival. Table 2 shows that there was no significant excess mortality in the irradiated series of either replicate, the only significant difference going in the opposite direction. An analysis of variance showed significant lack of homogeneity between the two replicates with respect to numbers of implants and live embryos; the reason for this is unknown. There was no evidence to suggest that the overall fertility of sons of irradiated males was reduced as a result of the irradiation.

Table 2. *Analysis of embryonic death and survival in fertility tests on sons of control and irradiated males, with replicates A and B kept separate. Three females dissected per tested male*

		Control totals	Irradiated totals	Mean difference (C − I) per male	t	P
Males tested	A	101	101			
	B	111	111			
Corpora lutea	A	2790	2713	+0·76	1·33	> 0·1
	B	2988	2970	+0·16	0·28	> 0·8
Implants	A	2237	2149	+0·87	1·52	> 0·1
	B	2228	2356	−1·15	2·26	< 0·05
Moles	A	212	213	−0·01	0·05	> 0·9
	B	210	235	−0·23	0·92	> 0·3
Live embryos	A	2012	1922	+0·89	1·50	> 0·1
	B	2006	2108	−0·92	1·58	> 0·1

4. DISCUSSION

The failure to find any significant induction of dominant lethals and translocations following 1200 r. γ-irradiation at low dose-rate is in striking contrast to the results of Lyon *et al.* (1964) with 1200 r. acute x-irradiation. They found that the number of live embryos per litter in the irradiated series averaged 1·01 less than in the controls, with a corresponding rise in numbers of dead implants. In addition,

3·5% of sons of irradiated males showed semi-sterility due to translocation heterozygosis, but only 0·2% of controls. All these differences were highly significant. This frequency of 3·5% semi-sterility in the x-irradiated series is also significantly higher than the 0·9% obtained in the present experiment with low intensity irradiation ($P = 0{\cdot}039$ by Fisher's exact treatment, with a one-tailed test). The higher yield with acute x-irradiation is supported by Searle's (1964) finding of semi-sterility in 7 out of 104 daughters (6·7%) of the same 1200 r. x-irradiated males.

A direct comparison of the findings in the present experiment and that of Lyon *et al.* suggests that high intensity x-irradiation is about four times as effective as low intensity γ-irradiation for the induction of translocations. A similar direct comparisons of extra embryonic lethality in the irradiated series of the two experiments, using live embryo/corpus luteum ratios, would suggest that the high intensity irradiation was about six times as effective as the low for the induction of dominant lethal mutations. Very little weight can be attached to either of these estimates because of the non-significant differences between control and irradiated series in the present experiment.

Russell (1963) believes that the dose-rate effect found with respect to specific locus mutations is most probably due to greater repair of premutational damage at low dose-rates, though Purdom & McSheehy (1963) consider that mechanisms based on differential radio-sensitivity of germ-cells cannot yet be ruled out. With chromosomal mutations, however, the concept of restitution would seem to be the most fruitful one for understanding the dose-rate effect. Reciprocal translocations are 'two-hit' aberrations, which can only be formed if the underlying events occur in the same nucleus and are close enough in time and space to allow the necessary exchange. As Neary *et al.* (1963) have pointed out, a long exposure-time makes the required interaction of the pair of events from two separate ionizing particles less likely, because the time interval between the two events tends to increase and recovery processes (leading to restitution) intervene. Thus with longer and longer exposure-times the square term in the relation between aberration yield (y) and dose (d)

$$y = K + \alpha d + \beta d^2$$

becomes smaller and smaller, while the linear term remains, since it represents aberrations due to two events caused by the same ionizing particle. Thus the yield approaches a lower limiting value at long exposure-times.

This is presumably the reason for the lowered yield of translocations at low dose-rates. The situation with respect to dominant lethals is more complicated. Lyon *et al.* (1964) have shown that dominant lethality resulting from spermatogonial irradiation is partly a secondary consequence of translocation induction, but their results suggested that there is some 'primary' dominant lethality as well. If this latter type is also caused by a 'two-hit' process, then the dose-rate effect with respect to dominant lethals would best be explained in terms of restitution, as with translocations. But if a 'one-hit' process is involved then other hypotheses,

invoking differential radio-sensitivity or more efficient restitution of breaks after chronic irradiation, would have to be considered. Only further experimental work can reveal the true state of affairs.

SUMMARY

1. F_1 (C3H♀ × 101♂) male mice were given 1200 r. ^{60}Co γ-irradiation over twelve weeks and mated twelve weeks after the end of irradiation. The incidences of foetal and neonatal lethality and of semi-sterility in their offspring were compared with those in controls.

2. Embryonic survival to 15 days in the irradiated series was 98·2% that of controls, while the incidence of semi-sterility was 0·9% compared with nil in the controls. Neither of these differences is significant.

3. Comparison of these results with the significant rates of induction of dominant lethals and translocations in a previous experiment, in which a dose of 1200 r. acute x-irradiation was given to males of the same hybrid stock, show the existence of a dose-rate effect. Its magnitude cannot at present be accurately estimated.

4. The reasons for this effect are discussed. It is concluded that the main cause of the lowered translocation yield is that restitution of breaks will be favoured at low dose-rates, with less opportunity for the formation of interchanges. This could partly account for the lowered rate of induction of dominant lethals as well, but a full explanation is not yet possible.

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REFERENCES

CARTER, T. C. & LYON, M. F. (1961). An attempt to estimate the induction by X-rays of recessive lethal and visible mutations in mice. *Genet. Res.* **2**, 296–305.

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.

LYON, M. F., PHILLIPS, R. J. S. & SEARLE, A. G. (1964). The overall rates of dominant and recessive lethal and visible mutation induced by spermatogonial x-irradiation of mice. *Genet. Res.* **5**, 448–467.

NEARY, G. J., SAVAGE, J. R. K., EVANS, H. J. & WHITTLE, J. (1963). Ultimate maximum values of the RBE of fast neutrons and gamma-rays for chromosome aberrations. *Int. J. Rad. Biol.* **6**, 127–136.

PHILLIPS, R. J. S. (1961). A comparison of mutation induced by acute X and chronic gamma irradiation of mice. *Brit. J. Radiol.* **34**, 261–264.

PURDOM, C. E. & MCSHEEHY, T. W. (1963). Dose-rate and the induction of mutation in *Drosophila*. *Int. J. Rad. Biol.* **3**, 265–275.

RUSSELL, W. L. (1963). The effect of radiation dose rate and fractionation on mutation in mice. Pp. 205–218 of *Repair from Genetic Radiation Damage*, ed. Sobels. Oxford: Pergamon.

SEARLE, A. G. (1964). Genetic effects of spermatogonial x-irradiation on productivity of F_1 female mice. *Mutation Research*, **1**, 99–108.

WELSHONS, W. J., GIBSON, B. H. & SCANDLYN, B. J. (1962). Slide processing for the examination of male mammalian meiotic chromosomes. *Stain Tech.* **37**, 1–5.