MUTAGENIC EFFECTS OF REPEATED SMALL RADIATION DOSES TO MOUSE SPERMATOGONIA

I. SPECIFIC-LOCUS MUTATION RATES

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SUMMARY

When male mice were given repeated small daily doses of 10 rad \( \gamma \)-rays at high dose rate (17 rad/min) the yield of specific-locus mutations was less than when the same total dose (600 rad) was given in a single exposure, and about the same as that after irradiation at low dose rate (0.008 rad/min).

However, when a similar total dose was split into doses of 50 rad at weekly intervals the results depended on dose rate. The mutation rate after 12 \( \times \) 50 rad X-rays at 66.70 rad/min was not significantly different from that after a single exposure, whereas the mutation rate after 12 \( \times \) 50 rad \( \gamma \)-rays at 0.06 rad/min was typical of low dose-rate irradiation. This confirms that the effect of dose rate on mutation rate does not depend on continuity or close spacing of the exposures. However, it is suggested that the “small dose” effect depends both on size of each dose and on close spacing of doses.

INTRODUCTION

Radiation delivered at a slow rate to mouse spermatogonia or oocytes produces fewer specific-locus mutations than does the same total dose delivered at a high rate (refs. 5–12). This raises the question whether a number of small radiation doses delivered at a high rate would also have less effect than the same total dose given in a single exposure. RUSSELL found that splitting a dose of 400 R to mouse oocytes into 8 fractions of 50 R at 75-min intervals considerably reduced the yield of specific-locus mutations, and also that a single dose of 50 R produced less effect than might have been expected by linear extrapolation from the results of higher doses.

In spermatogonia, irradiation with repeated small doses gives a yield both of dominant lethals and of cytologically scored translocations considerably lower than that obtained from a single dose of the same total size. In the case of translocations this could mean that two hits are required to produce an aberration. The evidence, however, is against this. A dose–response curve, plotted for 3, 6, 9 or 12 weeks' exposure to 10 rad/day, suggested that the first 3 weeks were giving a greater yield of
translocations than any succeeding 3-week period, and hence that the reduction of
yield was due to some change in sensitivity of the spermatogonial cell population with
repeated irradiation. It was of interest to find whether any comparable phenomena
also occurred in the case of specific-locus mutations.

In the experiments described here, male mice were given small doses of X- or
γ-rays at a high dose rate repeated at daily or weekly intervals, and the specific-locus
mutation rates among their offspring were compared with those resulting from the
same total dose in a single exposure at high rate, or in daily or weekly exposures at a
low rate.

MATERIALS AND METHODS

Two separate experiments are described. In both the tested males were of the
F₁ hybrid (C3H/HeH × 101/H) type usually used in this laboratory. At an appropriate
time after irradiation they were mated to females of the PT stock, homozygous for the
seven recessive genes a, b, c th, d, p, s and se. The mated pairs were left to breed as
long as they would, and young were scored for mutations when 2.5 weeks old.

Expt. 1

The males were divided into two groups and irradiated with a total dose of
about 600 rad γ-rays from a Co₆₀ source, at a dose rate of 17 rad/min. The first group
were given the whole dose in a single whole-body exposure at 12–13 weeks of age and
received about 620 rad, whereas the second group were given 60 doses of about 10.4
rad on five consecutive days each week for 12 consecutive weeks, beginning at 6 weeks
of age. Owing to decay of the source the average dose they received was only 610 rad.
The animals were then left for 12–17 weeks to recover fertility, before mating. Some
of the animals from this experiment were used for study of translocations.

Expt. 2

In this experiment animals were irradiated at weekly intervals, again up to a
total dose of 600 rad. One group were given 12 doses of 50 rad X-rays (250 kVp, h.v.l.
1.2 mm Cu, 60–70 rad/min) in 12 consecutive weeks, beginning at about 4 weeks of
age. The second group were exposed for one night each week to 50 rad γ-rays from
a Co₆₀ source. Owing to decay of the source the dose rate varied over the course of the
experiment from 0.07 to 0.05 rad/min, and the duration of exposure, needed to give
a dose of 50 rad, therefore ranged from 12 to 16 h. Both groups of animals were kept
for 7 weeks after the last exposure before mating.

In addition, we also report in this paper some further data from the experiment
of PHILLIPS, in which she compared the specific-locus mutation rates after doses of
600 rad given either in a single exposure to X-rays (250 kVp, h.v.l. 1.2 mm Cu) at
60–70 rad/min, or in 90 consecutive daily exposures to Co₆₀ γ-rays at about 0.008
rad/min.

RESULTS

The results from the two new experiments (treatments 1 and 2, and 4 and 5)
together with the total data from the experiment of PHILLIPS (treatments 3 and 6)

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RADIATION EFFECTS ON MOUSE SPERMATOGONIA. I.

TABLE I

SPECIFIC LOCUS MUTATION RATES AFTER 600 rad X- or γ-RAYS TO MALE MICE IN SINGLE OR REPEATED DOSES

<table>
<thead>
<tr>
<th>Treatment No.</th>
<th>Radiation type</th>
<th>Interval and number of exposures</th>
<th>Dose rate (rad/min)</th>
<th>Total offspring</th>
<th>Mutants</th>
<th>Mutation rate per locus (× 10⁻⁵)</th>
<th>Limits a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>γ</td>
<td>Single</td>
<td>17</td>
<td>12021</td>
<td>11</td>
<td>13.1</td>
<td>7.6, 24.7</td>
</tr>
<tr>
<td>2</td>
<td>γ</td>
<td>Daily (60)</td>
<td>17</td>
<td>23982</td>
<td>7</td>
<td>4.2</td>
<td>1.7, 8.6</td>
</tr>
<tr>
<td>3</td>
<td>γ</td>
<td>Daily (90)</td>
<td>0.008</td>
<td>22682</td>
<td>5 b</td>
<td>3.2</td>
<td>1.0, 7.4</td>
</tr>
<tr>
<td>4</td>
<td>γ</td>
<td>Weekly (12)</td>
<td>0.05-0.07</td>
<td>22816</td>
<td>10</td>
<td>6.3</td>
<td>3.0, 11.5</td>
</tr>
<tr>
<td>5</td>
<td>X</td>
<td>Weekly (12)</td>
<td>60-70</td>
<td>18119</td>
<td>16</td>
<td>12.6</td>
<td>7.9, 21.3</td>
</tr>
<tr>
<td>6</td>
<td>X</td>
<td>Single</td>
<td>60-70</td>
<td>11138</td>
<td>12 b</td>
<td>15.4</td>
<td>9.1, 28.2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>157421</td>
<td>11 c</td>
<td>1.0</td>
<td>0.50, 1.79</td>
</tr>
</tbody>
</table>

a 95% fiducial limits of mutation rate × 10⁵.

b Includes data of PHILLIPS a.

c Includes data of CARTER et al. 1.

are summarized in Table I. Also given are the total control data, collected in this laboratory to date, including the data given by CARTER et al. 1. The mutation rate of 1.0 ± 10⁻⁵ per locus in these data compares with RUSSELL's 8 figure of 0.8 ± 10⁻⁵ per locus calculated from 28 mutations out of 531500 offspring.

The mutation rates from the two sets of single exposures, to X- or γ-rays (treatments 1 and 6), were both close to that which RUSSELL 8 found after single exposures at high dose rates (13.3 ± 10⁻⁵). By contrast, when 600 rad γ-rays were given in 60 daily doses of 10 rad, still at the high dose rate of 17 rad/min (treatment 2) the mutation rate of 4.2 ± 10⁻⁵ per locus was only about one-third of that after the comparable single exposures, and was close to that found after the low dose-rate irradiation at 0.008 rad/min. Thus, repeated small doses produced less effect than a single dose of the same size and the reduction in yield is of the same general order as was found in the earlier work with translocations.

When the small doses were given at weekly rather than daily intervals, however, the results were quite different. 12 doses of 50 rad X-rays one week apart (treatment 5) yielded a mutation rate (12.6 ± 10⁻⁵ per locus) nearly as high as that from a single exposure at the same rate (60-70 rad/min) and much higher than that from 12 weekly exposures to a low dose rate (0.05-0.07 rad/min) of γ-rays. The mutation rate from this last treatment regime (No. 4) was somewhat higher than that after daily irradiation, either at low or at high dose rate, but not significantly so (χ² 1 = 1.64, for heterogeneity of treatments 3 and 4).

The spectra of mutations at the various loci are shown in Table II. In addition after treatment 1 there was one mutant which was excluded from the calculations. This was a mottled mouse which proved to be a gonosomal mosaic for a mutation to a. It was assumed that the mutation had not occurred in the spermatogonia of the father but in the early development of the mutant itself, and this was the reason for excluding it. A dominant visible mutation (W allele) first found in the F₂ generation after treatment 2 (i.e. among the progeny of a mutant) was also excluded.

Although the absolute numbers of mutations are small it seems that mutation at each locus is reduced after treatment regimes giving a low mutation rate (Nos. 2-4), as demonstrated by RUSSELL 8. Moreover, although the numbers are extremely small,
TABLE II
SPECTRUM OF SPECIFIC-LOCUS MUTATIONS AFTER VARIOUS RADIATION TREATMENTS

<table>
<thead>
<tr>
<th>Treatment No.</th>
<th>Locus</th>
<th>Dominant visible</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>a-b</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>c</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>d</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>e</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>f</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>g</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>h</td>
<td></td>
</tr>
<tr>
<td>2, 3 and 4 (low)</td>
<td>i</td>
<td></td>
</tr>
<tr>
<td>1, 5 and 6 (high)</td>
<td>j</td>
<td></td>
</tr>
</tbody>
</table>

a Includes a cluster of 2.

dominant visible mutations also were fewer after the repeated and low dose-rate regimes.

As many as possible of the mutants were tested for viability (Table III). There was a suggestion, significant at the 5% level, that a higher proportion of mutations were lethal after treatments 1, 5 and 6. However, it is very difficult to know what importance, if any, to attach to this, since there were obviously marked differences among loci in proportion of mutations that were lethal, and moreover, mutations at the d and s loci, which are most often lethal, seemed somewhat less common after treatments 2-4.

Two of the three simultaneous d and se mutations were viable, and therefore, according to L. B. Russell's interpretation, the result of double nondisjunction, and the third was lethal and presumably due to a small deletion. All of the d mutants

TABLE III
VIABILITY OF MUTATIONS AFTER VARIOUS RADIATION TREATMENTS

<table>
<thead>
<tr>
<th>Treatment No.</th>
<th>V, viable; L, lethal.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

a Includes a cluster of 2.

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showed the neuromuscular abnormality typical of lethal $d$ mutants, and called by RUSSELL $d^{op}$. Two were a cluster with a new type of $d$ mutation, designated slight-dilution, $d^s$, in which the coat colour was only slightly altered, but the neuromuscular abnormality was typical.

**DISCUSSION**

In these experiments the specific-locus mutation rate after daily repeated small radiation doses at high dose rate was clearly less than after a single exposure of comparable total size. Thus, this phenomenon of a reduced mutagenic effect of repeated small doses, previously found with translocations, is not limited to them. This means that in seeking an explanation there is no need to invoke any mechanism peculiar to translocations, such as a two-hit effect.

A main question to be answered is whether each single one of the many small doses has a low effect, possibly because of some repair process, or whether repetition of doses is important, perhaps by causing some change in the spermatogonial cell population. When doses of 50 rad X-rays at high rate were given at weekly intervals, the mutation rate was very little less than that after a single exposure. Two main explanations seem possible here. Either a dose of 50 rad is not sufficiently small to give a "small dose" effect, or else the interval between doses is important. If the latter, then this would suggest that changes in the spermatogonial cell population were involved, either through cell selection or some type of repair.

In female mice a dose of 50 R produced a "small dose" effect (i.e. a lower than expected mutation rate) either when given as a single dose or when 8 repeated doses were given at 75-min intervals. It is possible that males and females differ in this respect, and that 50 rad does not qualify as a "small dose" in the male. However, it is known that repeated daily doses of 60 rad to spermatogonia do give a reduced yield of translocations, although the reduction was less than when the size of each dose was 10 rad.

This might suggest that the absence of any marked reduction of specific-locus-mutation rate after the weekly doses of 50 rad was due to the long intervals between the doses. Against this, however, is the fact that, again with translocations, $12 \times 50$ rad gave a reduced yield, whether the doses were given daily or weekly, with the weekly spaced doses only slightly more effective. On balance it seems probable that the size of each dose and the interval both play a part in determining the effect of repeated small doses. Also, it seems likely that in the male the reduction in yield due to small size of a dose of 50 rad is less than that in the female. This calls to mind the more marked effect of dose rate in the female and RUSSELL's suggestion that the same mechanism might be responsible for the reduced effect of low dose rates and of small doses.

If interval between doses is important at all in determining the reduced effect of small doses it raises the question whether the dose-rate effect could be due to a change in sensitivity of the spermatogonial population with prolonged irradiation as well as simply to the low dose rate itself. RUSSELL tested this point by plotting dose-response curves with doses delivered at both 0.009 and 0.001 R/min and found no evidence of the nonlinearity which would be expected if spermatogonial sensitivity changed with time during the prolonged exposure. Moreover, a dose of 600 R delivered at 0.8 R/min gave a low mutation rate and here the duration of exposure seems too short to produce any change in cell population. In our experiments we have seen that 12

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doses of 50 rad γ-rays at 0.05–0.07 rad/min given one week apart yield a much lower mutation rate than comparable doses given at 60–70 rad/min. This shows clearly that low dose-rate irradiation does not have to be given continuously or at very short intervals in order to give a reduced mutagenic effect. However, the mutation rate with weekly doses at low dose rate seemed higher than after comparable daily or continuous doses. Although the difference was not statistically significant this does seem to raise the question whether, after the more closely spaced doses, the reduction in mutation rate might only partly be due to the low dose rate and also partly to a repetition effect.

A final point concerns the small difference between the results of our two single-dose treatments. The 600 rad γ-rays at 17 rad/min were slightly less effective than X-rays at 60–70 rad/min. A comparable difference was found by Russell12 between γ-rays at 24 R/min and X-rays at 90 R/min. It is not possible to tell from either Russell’s work or our own (a) whether the difference is a real or a chance one and (b) if real, whether it is due to a difference in dose rate or in radiation quality. If the latter, then our results confirm those of Russell12 in giving a relative biological effectiveness (RBE) of 0.8 for γ- versus X-radiation.

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REFERENCES