The Rejoining Time of Chromatid Breaks Induced by Gamma Radiation in *Vicia faba* Root Tips at 3°C.

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**ABSTRACT**

The observation was made previously that the reduction in radiosensitivity in *Vicia faba* (as measured by postirradiation root growth) by prolonging the exposure time from about 10 minutes to 24 hours is much less marked at 3°C. than at 19°C. If chromosome damage is mainly responsible for the reduced root growth, this observation might be explained by a smaller drop in the “two-hit” aberration component, resulting from an increased time for which breaks are available for rejoining at 3°C.

This hypothesis was tested by comparing chromatid aberration frequencies in root meristem cells produced by 105 rads of 60Co γ rays, given at dose rates of 19.4 and 0.073 rads per minute. Beans were maintained in aerated water at 2°C. prior to and during irradiation, and at this temperature the rate of development of cells was such that the two different exposure times both occupied a period during which the cell sensitivity was approximately constant. Immediately subsequent to irradiation, the roots were returned to 19°C. and examined cytologically.

All chromatid aberrations were less frequent after low dose rate treatment, but only the chromatid interchange reduction was significant.

The average time for which breaks are available for reunion, calculated from Lea's G function, was found to be 12 hours (95 per cent C.L. 6 to 24 hours).

**INTRODUCTION**

The curious observation was made by one of us (8, 9) that the dependence of the radiosensitivity of broad bean roots (*Vicia faba*) on temperature was much more marked for long gamma radiation exposures (24 hours), than for short exposures (10 minutes). The index of radiation effect was the reduction in the total growth of the primary root in the period of 10 days following irradiation. The ratio of radiosensitivities in air-saturated water at 3°C. and 19°C. was 1.56 for a long exposure but only 1.16 for a short exposure. If allowance is made for the fact that there is a greater concentration of oxygen in air-saturated water at 3°C. than at 19°C. (9.4 cc. O₂ per litre and 6.5 cc. O₂ per litre respectively), the ratio of radiosensitivities at the two temperatures with the same concentration of oxygen would be 1.35 for a long exposure and 0.98 for a short one. Thus for a short exposure there is no intrinsic dependence on temperature whereas the long exposure is more effective at the lower temperature.

From the same experimental data, the ratio of radiosensitivities at a given temperature for short and long exposures was also evaluated. The results obtained, using doses which reduced the growth rate over the 10 day period to roughly one-half of normal, were 1.79 at 19°C. and 1.33 at 3°C.: the precise figures would depend on the level of injury considered.

Some decrease in radiosensitivity on prolonging the exposure time could be expected on the theory that chromosome damage in the meristematic cells is an important factor in the reduction of root growth by radiation (5). If the average duration for which a chromosome break is available for rejoining at 19°C. were considerably longer than the duration of the short exposure (10 minutes), but considerably shorter than the duration of the long exposure (24 hours), a marked drop in the “two-hit” component of chromosome damage would be expected with the long exposure at this temperature. The smaller fall-off in radiosensitivity at 3°C. would imply a smaller drop in the...
“two-hit” component of chromosome damage, and therefore a considerably increased rejoining lifetime of breaks at this temperature; such an increase has been shown to occur in Vicia seed irradiated at low temperature (15). The rejoining lifetime of breaks in the growing root has not been measured at room temperature because the variation in sensitivity throughout the mitotic cycle, and the rapidity of cell development, render the interpretation of dose fractionation and protraction experiments difficult. Indirect evidence from the dependence of root growth inhibition on exposure time (5; Neary, unpublished), suggests that the rejoining lifetime of chromosome breaks in roots at 19°C. is of the same order as the value determined cytologically in seed at 25°C., namely, about 1 hour (14).

It seemed possible that the existence of an increased rejoining lifetime of breaks at 3°C., indicated from the root growth studies above, could be checked cytologically. Savage and Evans (11) have shown that the duration of the mitotic cycle at 3°C. is some ten times greater than at 19°C., i.e., about 240 hours. From the observed variation in sensitivity to breakage at different parts of the mitotic cycle at 19°C., it could be expected that at 3°C., the sensitivity of a cell would remain approximately constant for several hours at least. It should thus be possible to confirm cytologically, by fractionation or protraction experiments, that the rejoining lifetime of breaks is considerably greater than 1 hour at this temperature. Accordingly, the number of chromatid aberrations in the period of peak sensitivity produced at 3°C. by radiation doses given in about 5 minutes and 24 hours has been compared.

**Materials and Methods**

The material used in these experiments was cells from the terminal root meristem of Vicia faba (var. Sutton's prolific longpod). Details of culture and treatment prior to experimentation have been described in detail elsewhere (3).

For the main experiment, three batches of beans were placed in aerated tap water, maintained at 2.0 ± 1.0°C. After 9 days, one batch was subjected to 105 rads of 60Co γ rays delivered in 24 hours (dose rate 0.073 rad per minute). At the end of this period, a second batch was given 105 rads in 5.42 minutes (dose rate 19.4 rads per minute). The roots were immersed in air-saturated water at 2°C. throughout both irradiations. All three batches were then returned simultaneously to aerated tap water at 19°C., and fixations made at hourly intervals up to 7 hours. The third, unirradiated batch were used as controls to check the recovery of mitotic activity on return to 19°C.

The two batches of irradiated roots were fixed in Ford's modification of Flemming fluid, and the control batch in 1:3 acetic acid-alcohol mixture. Subsequent to Feulgen staining, squashes were made permanent by the dry ice technique (2).

At every fixation time, 25 metaphase cells on each of three coded and randomised slides were fully analysed for chromatid type aberrations.

### RESULTS

#### A. Preliminary Work

The rates of cell development at 3°C. were determined by the use of

<table>
<thead>
<tr>
<th>Aberration type</th>
<th>Number of cells scored at each fixation time</th>
<th>Low dose rate</th>
<th></th>
<th>High dose rate</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fixation (hrs. from irradiation)</td>
<td></td>
<td></td>
<td>fixation (hrs. from irradiation)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Chromatid break</td>
<td>75</td>
<td>0.26</td>
<td>0.32</td>
<td>0.09</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.05</td>
<td>±0.10</td>
<td>±0.04</td>
<td>±0.10</td>
</tr>
<tr>
<td>Isochromatid break</td>
<td>75</td>
<td>0.13</td>
<td>0.09</td>
<td>0.11</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.02</td>
<td>±0.04</td>
<td>±0.05</td>
<td>±0.02</td>
</tr>
<tr>
<td>Chromatid interchange</td>
<td>75</td>
<td>0.11</td>
<td>0.17</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.08</td>
<td>±0.11</td>
<td>±0.02</td>
<td>±0.04</td>
</tr>
<tr>
<td>Chromatid interchange</td>
<td>125</td>
<td>0.21</td>
<td>0.18</td>
<td>0.16</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>±0.06</td>
<td>±0.04</td>
<td>±0.02</td>
<td>±0.03</td>
</tr>
</tbody>
</table>
colchicine, and the details of the method have been published elsewhere (11).

It was shown that the initial response of different stages in interphase when the temperature is changed from 19° to 3°C. is not uniform, and a time equivalent to one complete passage through the mitotic cycle is required before equilibrium in cell rate is achieved. The total cycle time is about 240 hours, (compare about 25 hours at 19°C.), so that cells have been slowed by a factor of about 10. At this rate of development, a period of 24 hours is equivalent to a period of about 2 hours at 19°C., so that a 24 hour irradiation will occupy a period during which cell sensitivity will remain approximately constant.

These rates of cell development were confirmed by the use of an irradiation method. In one experiment, roots were irradiated at 19°C. with 122 rads 60°Co γ rays and transferred immediately to water at 3°C. The peak frequency of chromatid interchanges, which at 19°C. usually occurs about 3 to 4 hours after irradiation, was delayed until 24 to 30 hours, indicating that cell development was retarded by a factor of about 8.

In a second experiment, roots maintained at 3°C. for 9 days, were subjected to 105 rads 60°Co γ rays in about 5 minutes at 3°C. and then maintained in the cold. Unambiguous chromatid aberrations (interchanges) which at 19°C. begin to appear about 1 1/2 to 2 hours after irradiation (10; Revell, private communication) did not appear until 12 hours and peak frequency had still not been reached at 24 hours, confirming a very slow movement of cells.

B. Main Experiment.—The mean yield of aberrations per cell together with standard errors for the four main types of chromatid damage are given in Table I and Figs. 1 to 4. In calculating the standard errors, since the sample numbers are small, the standard deviation was derived from the range (13). At 19°C., prophase lasts from 1 1/2 to 2 hours, so that on transferring roots from 2° to 19°C., any cells appearing in metaphase before this interval has elapsed, must have entered mitosis before the temperature change. Any
aberrations occurring in these cells after the low dose rate will not have been produced by the full 105 rads, since true chromatid breaks cannot be produced in cells irradiated at prophase (10). Therefore, only metaphase cells from 3 hours after irradiation and onward have been used for the purpose of analysis.

The data were subjected to analysis of variance, with and without square root transformation. The significance of the difference between the aberration grand means, over all fixation times, is given in Table II. The values in the long exposure series are all lower than those in the short exposure series, but the difference is significant ($p = 0.01 - 0.001$) only for chromatid interchanges.

A tendency for a slight displacement to later times of the peak aberration frequencies produced by the high dose rate suggested the possibility of differential mitotic delay at the two dose rates. We have shown that for doses of about 100 rads of gamma rays, given in 5 minutes at 19°C, the absolute mitotic delay in the first 6 hours after irradiation is not more than about 2 hours (10, 4). To allow for the possibility of differential delay, the interchange data at both dose rates were also compared assuming an average difference in delay of 1 hour, (there being no difference in the shape of the curves). No change in the significance level of the difference between the mean aberration yields was found ($P < 0.01$).

An apparent increase in the frequency of complex interchanges; i.e. exchanges involving three or more chromosomes, but excluding isochromatid/chromatid interchanges, was observed when the data were compared with that from similar doses given at 19°C. At low dose rate there were 8 complex interchanges in 625 cells, and at high dose rate 9 in 625 cells, compared with 1 in 225 cells at 19°C. after 121 rads of $^{60}$Co $\gamma$ rays at a dose rate of 22 rads per minute.

**Table II**

<table>
<thead>
<tr>
<th>Aberration type</th>
<th>High dose rate $x_1$</th>
<th>Low dose rate $x_2$</th>
<th>$(x_1 - x_2)$</th>
<th>S.E.</th>
<th>$t$</th>
<th>Degrees of freedom</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatid break</td>
<td>0.20</td>
<td>0.18</td>
<td>0.02</td>
<td>0.04</td>
<td>0.56</td>
<td>748</td>
<td>0.6-0.5</td>
</tr>
<tr>
<td>Isochromatid break</td>
<td>0.14</td>
<td>0.11</td>
<td>0.03</td>
<td>0.03</td>
<td>0.91</td>
<td>748</td>
<td>0.4-0.3</td>
</tr>
<tr>
<td>Chromatid intrachange</td>
<td>0.09</td>
<td>0.08</td>
<td>0.01</td>
<td>0.03</td>
<td>0.37</td>
<td>748</td>
<td>0.8-0.7</td>
</tr>
<tr>
<td>Chromatid interchange</td>
<td>0.26</td>
<td>0.17</td>
<td>0.09</td>
<td>0.03</td>
<td>3.29</td>
<td>1248</td>
<td>0.01-0.001</td>
</tr>
</tbody>
</table>

**Discussion**

The main finding of this experiment, namely the reduction of "two-hit" aberration types with an increase of exposure time, is in agreement with the results of previous work on a variety of materials.

The generally accepted explanation of this reduction is that primary "breaks" produced by ionizing radiation are available for reunion only for a limited time, of mean duration $\tau$. Extension of the time taken to administer a given dose reduces the number of contemporaneous breaks, thus lowering the probability of occurrence of interchange. This interpretation is, in general, supported by the results of fractionated dose studies.

On the classical view of breakage and reunion, it is possible to obtain an estimate of $\tau$. Briefly, according to Lea (7) the number of interchanges produced in a cell by a dose $D = IT$ is proportional to $G \cdot D^2$. 

![Graph illustrating Chromatid interchanges per cell produced by 105 rads $^{60}$Co $\gamma$ rays.](image)

FIG. 4. Chromatid interchanges per cell produced by 105 rads $^{60}$Co $\gamma$ rays. $\circ$, 0.073 rad per minute; $\bullet$, 19.4 rads per minute.
In which \( D = \) the dose
\( I = \) the intensity of the dose (dose rate)
and \( T = \) the time over which the dose was administered.

If \( D \) remains constant, and \( I \) is varied, the yield of interchanges should be proportional to \( G \); i.e., a graph of the number of “two-hit” interchanges against the duration of irradiation, \( T \), should be identical in shape with the function \( G \). This function for various values of \( T/\tau \) is tabulated by Lea (7) and therefore, by determining the best fitting \( G \) curve to a graph of “two-hit” interchanges per cell against the duration of exposure, an estimate of \( \tau \) may be obtained.

We may attempt to apply the theory to our \( Vicia \) chromatid interchange data. Fig. 5 shows the mean frequency of chromatid interchange per cell, (based on fixation times 4 to 7 hours) for low and high dose rates, plotted against the duration of the dose \( T \), together with a curve of \( G \) assuming \( \tau \) to be 1 hour, cf. the value at 19°C. (14).

It will be seen that the number of interchanges per cell at the low dose rate is much higher than would be expected for a \( \tau \) of this value, and therefore \( \tau \) must be much greater than 1 hour. Now, from the theory (7), as the ratio \( T/\tau \) approaches zero, \( G \) tends to unity. We know that \( \tau \) exceeds 1 hour and that \( T \) for the high dose was about 5 minutes. This gives a maximum value for \( T/\tau \) of 0.08. As this value is approaching zero, \( G \) for the short exposure is very close to unity. The value of \( G \) for the long exposure is then very close to the ratio:

\[
\frac{\text{Mean interchanges per cell at low dose rate}}{\text{Mean interchanges per cell at high dose rate}}
\]

and its limits may be calculated. This ratio is 0.567 (95 per cent confidence limits 0.401 and 0.734), corresponding to \( T/\tau \approx 2.00 \). Since \( T = 24 \) hours, \( \tau = 12 \) hours (95 per cent C.L. 6.6 to 24 hours). The confidence limits are large because the variation between fixation times has been introduced into the error of the mean, in addition to between-slide variation.

There are certain difficulties to be considered in this method of deriving \( \tau \).

First, the theory only applies to interchanges arising from two separate “hits” or events, but it is known that a proportion of chromatid interchanges are the result of one event. If, under the present experimental conditions, this component is considerable at high dose rates, and if, by analogy with other “one-hit” aberrations, it is unaffected by a reduction in dose rate, the curve of interchanges will only fall until the “one-hit” interchange value is reached, and thereafter will remain constant. At 19°C. in this material, with doses of about 100 rads in about 4 minutes about 14 per cent of the interchanges are “one-hit” (Neary and Evans, data to be published). We have no information about the value of this component at 3°C., but employing the 19°C. value, “one-hit” inter-

![Fig. 5. Mean numbers of chromatid interchanges per cell (fixation times 4 to 7 hours) for dose rates of 0.073 rad per minute O, and 19.4 rads per minute O, together with a curve of G assuming “breaks” to remain open for an average time of 1 hour.](image-url)
changes would contribute about 0.04 per cell to the total interchanges. This is not sufficient to account for the high value observed at low dose rate.

Second, the function $G$ was shown to hold for *Tradescantia* microspores only for values of $T$ less than 30 minutes ($r = 4$ minutes) (1), but at greater times the observed value was higher than expected. Since the “one-hit” component had been eliminated from the data, this departure was attributed to the presence of at least two values for $r$, one measurable in minutes and the other in hours. More recent work (6, 16) has given larger values for $r$ in *Tradescantia* (about 20 minutes), so that at present it is uncertain over what values of $T$ the simple $G$ function will apply. If a reduction in temperature increases the value of $r$ (1, 12) one would expect the $G$ curve to apply over a greater range of $T$ than at higher temperatures. However, as there are no intermediate points on our curve, there is no means of assessing the extent of this departure at 24 hours.

Third, there is also the possibility of an intrinsic dose rate effect on primary breakage, which would modify the response curve for interchanges. It may be noted that even the “one-hit” aberrations, which, on the classical view, should be independent of dose rate and exposure time, were in fact lower at the low dose rate, though not significantly so. Previously, an effect of dose rate on primary breakage was looked for by comparing root growth inhibition after a continuous exposure of 24 hours and after a large number of small fractional doses at high dose rate extending over a total period of 24 hours. No significant difference was found. Any effect of dose rate in a cytological experiment would be eliminated by fractionating the dose at constant dose rate, and such experiments are at present in progress. These will give another estimate of $r$.

A rough value of $r$ may also be derived from the data on root growth inhibition given in the Introduction. For this purpose, root growth inhibition is assumed to be determined entirely by chromosome damage. The effective chromosome damage will be a suitably weighted combination of the “one-hit” and “two-hit” components of the form $\alpha D + \beta GD^2$. For short exposures, at either 19°C or 3°C, $G$ is nearly unity. There is no intrinsic temperature dependence for short exposures, so $\alpha$ and $\beta$ are independent of temperature. For a long exposure (24 hours) at 19°C, the damage is nearly all “one-hit” (5) and $G$ is zero. The ratio of the radiosensitivity for short and long exposures at 19°C is 1.79 at a certain level of damage;

$$\alpha D + \beta D^2 = 1.79\alpha D$$ (1).

The ratio of radiosensitivity after short and long exposures at 3°C is 1.33 at the same level of damage,

$$\alpha D + \beta D^2 = 1.33\alpha D + \beta G(1.33D)^2$$ (2)

in which $G$ is the value of the function for a 24 hour exposure at 3°C. From (1) and (2)

$$(1.33\alpha D) + \beta G(1.33D)^2 = 1.79\alpha D$$ (3).

Therefore from (1) and (3) respectively, we have

$$\beta D^2 = 0.79 \alpha D$$ and $$\beta G(1.33D)^2 = 0.40\alpha D$$

therefore $$G = (0.46/0.79) \times (1.33)^2 = 0.329$$

therefore $$\frac{T}{r} = 4.83$$ and since $T = 24$ hours,

$$r = 4.9$$ hours.

This estimate, though very approximate, supports the conclusion that the mean lifetime of breaks at 3°C is appreciably longer than 1 hour.

The authors would like to thank Miss S. M. Tonkinson, Mrs. S. M. Wells, and Mr. T. R. Bigger for valuable technical assistance, and Mr. D. Westgarth for help with the statistical analyses.

**BIBLIOGRAPHY**