RELATIVE BIOLOGICAL EFFICIENCY OF 14.1 MeV FAST NEUTRONS
AND ^{137}Cs GAMMA RAYS IN THE STAMEN HAIRS OF
TRADESCANTIA REFLEXA RAFIN.¹⁾

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Most species of the genus *Tradescantia* have many hairs on their stamen filaments. Each stamen hair is composed of a single chain of cells, and its development depends principally on the repeated division of the terminal cell. Thus the stamen hairs of this genus can be regarded as an essentially single-meristematic-cell system as demonstrated by Ichikawa and Sparrow (1967b), and can be used as an excellent plant material for radiobiological studies at the single-cell level (Davies 1963; Alvarez and Sparrow 1965; Nayar and Sparrow 1967; Ichikawa and Sparrow 1967a, 1967b, 1968, 1969; Ichikawa 1968; Ichikawa *et al.* 1969).

The present study was undertaken to determine the relative biological efficiency (RBE) of 14.1 MeV fast neutrons as compared with ^{137}Cs gamma rays in causing loss of reproductive integrity and in inducing somatic mutations in *Tradescantia* stamen hairs. The main reason why this study was carried out is that extremely high RBE values of 10 to 50 sometimes more than 100 have been reported in plant materials for fast neutrons or other heavy particles by many authors (Matsumura *et al.* 1963; Neary *et al.* 1963; Smith *et al.* 1964; Matsumura 1966 and many others, see Discussion). The RBE values reported in animals and microorganisms are generally much closer to 1 (at most 5) (see Kondo 1964). It should be remembered that most of the extremely high RBE values in plants have been obtained from irradiation of dry seeds containing a small amount of water (about 8 to 15 percent). It is generally accepted that water content modifies the sensitivity to X rays or gamma rays greatly but that to neutrons relatively slightly (Ehrenberg *et al.* 1952; Ehrenberg and Nybom 1954; Ehrenberg 1955; Ikushima 1968; Fujii 1969). The present RBE study in the single meristematic cells of actively growing stamen hairs, which contain a comparable amount of water with other organisms, will offer information to help resolve the above discrepancy reported between plants and other organisms.

MATERIAL AND METHODS

A tetraploid *Tradescantia* clone (KU 7, $2n=24$) was used in the present investigation. This clone was recently isolated as a heterozygote for flower color (blue/pink, blue being

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dominant) from the clone of *T. reflexa* Rafin. (4x) which had been kept in this laboratory for many years, and has chromosomes which are about as large as those of *T. virginiana* (see Ichikawa and Sparrow 1967b for *T. virginiana* chromosomes). Each inflorescence of the KU 7 clone is composed of at least 50 buds, one or two of which bloom daily. Each flower has six stamens and each stamen bears about 70 hairs. Each hair is a single chain of cells and the average numbers of cells per hair are 32.0, 28.3 and 23.4 at the basal, middle and distal thirds of the stamen filament, respectively.

Three potted plants with several inflorescences of flowering size were selected and were exposed to fast neutrons or gamma rays or used as control. Five inflorescences of Plant 1 were exposed to 14.1 MeV fast neutrons for 13 min, and three inflorescences of Plant 2 were irradiated with ^{137}Cs gamma rays for 8 min. Each young inflorescence, being composed of many buds, had a thickness of about 6 mm at the time of irradiation. Thus, in the fast neutron treatments in which the target distances were quite short, *i. e.*, 6.2 to 11.8 cm, the absorbed doses were calculated for each flower examined based on the simultaneous dosimetry with sulfur placed between inflorescences and based on the position of each flower bud at the time of irradiation. The calculated absorbed doses of fast neutrons ($\text{n/cm}^2 \times d_f$; $d_f = 6.68 \times 10^{-9}$) ranged from 37 to 141 rads (Table 1). In the gamma-ray treatments, on the other hand, the target distances were fairly long (63.2 to 106.8 cm), thus the doses were calculated based only on the simultaneous measurements with Toshiba's glass dosimeters placed on each inflorescence. The dose range of gamma rays was 144 to 362 rads (Table 1). One inflorescence of Plant 3 was used as control. All the irradiation treatments were carried out at the National Institute of Genetics, Misima, on December 13, 1968.

Table 1. Irradiation treatments with 14.1 MeV fast neutrons and ^{137}Cs gamma rays

Plant no.	Inflorescence no.	Radiation	Target distance* (cm)	Exposure time (min)	Dose (rads)
1	1	14.1 MeV neutrons	11.8	13	37-38**
	2	"	10.5	"	47-49**
	3	"	9.3	"	59-62**
	4	"	7.5	"	91-96**
	5	"	6.2	"	132-141**
2	6	^{137}Cs gamma rays	106.8	8	144
	7	"	79.6	"	251
	8	"	63.2	"	362
3	9	—	—	—	0

* Measured at the center of inflorescence.

** Calculated for each flower examined based on the position of each flower bud in the inflorescence at the time of irradiation (see text).

The irradiated and control plants were immediately carried back to Kyoto and were grown in a heated greenhouse in which the temperature ranged from 17 to 27°C and day length was kept at about 18 hrs using fluorescent lamps and tungsten bulbs at evening. Observations of stamen hairs were continued for 19 days after irradiation on

one or two flowers from each inflorescence. The number of stamen hairs was counted for each stamen and the number of cells per hair was counted on 24 randomly selected stamen hairs (eight each from the basal, middle and distal thirds of the stamen). The positions of all somatic mutant (pink or colorless) cells that appeared in the terminal 20 cell positions of each hair were also recorded. Moreover, in order to calculate the frequency of mutant cells in the hair cell population, the cell numbers were also recorded for all of the hairs which had mutant cells and were composed of less than 20 cells.

RESULTS

The effects of radiation treatments were first observed eight days after irradiation. This indicated that the development of the stamen hairs had been completed seven days before flowering. The radiation effects, *i. e.*, reduction of cell number per hair, induction of somatic mutations *etc.*, increased up to postirradiation days 11 or 12. However, the maximum radiation effects could not be observed for the highest dose of fast neutrons (Inflorescence No. 5) because the development of flowers had been severely disturbed with this dose and consequently no flower opened for the period (after day 13) when the maximum effects were expected to be observed (the maximum effects can be

Fig. 1. Average numbers of cells per hair at 11 or 12 days after irradiations with 14.1 MeV fast neutrons and ¹³⁷Cs gamma rays. The point for the highest dose of fast neutrons was not used for drawing the line (see the first paragraph of Results).

Fig. 2. Survival curves of the meristematic hair cells after treatments with 14.1 MeV fast neutrons and ^{137}Cs gamma rays. The point for the highest dose of fast neutrons was not used for drawing the line (see the first paragraph of Results).

observed later with higher doses (see Ichikawa and Sparrow 1967a, 1967b). Therefore, in this paper, the radiation effects observed on postirradiation days 11 or 12 (the maximum effects for all inflorescences except the effects for No. 5) were compared between doses or between fast neutrons and gamma rays. The data for the highest dose of fast neutrons were thus not used for analyses although the values on day 12 were plotted in figures.

The average numbers of cells per hair at 11 or 12 days after irradiation are plotted in Fig. 1 against log dose. The line drawn for fast neutrons is the one that fits best for the four lower doses ignoring the point for the highest dose. The line for gamma rays is the best fit through the three points plotted and, at the same time, is parallel to the line for fast neutrons. From the two parallel lines, it can be determined that 14.1 MeV fast neutrons are 3.95 times more efficient than ^{137}Cs gamma rays in reducing cell number per hair, *i. e.*, the RBE value is 3.95.

From the study of variation in the number of cells per stamen hair in control

Fig. 3. Postirradiation growth of hairs (increase in hair cell number) after treatments with 14.1 MeV fast neutrons and ^{137}Cs gamma rays. The point for the highest dose of fast neutrons was not used for drawing the line (see the first paragraph of Results).

flowers, the following three standards could be set to distinguish the hairs whose reproductive integrity had been lost or impaired from those retaining normal reproductive integrity. Thus, the hairs composed of 23 or less, 22 or less and 17 or less cells in the basal, middle and distal thirds of the stamen, respectively, were regarded as those which had lost their normal reproductive integrity. The validity of setting such standards has been discussed elsewhere (Ichikawa and Sparrow 1967a; Ichikawa *et al.* 1969). When those hairs as defined above are regarded as non-survivors, two survival curves are obtained, one for fast neutrons and the other for gamma rays, as seen in Fig. 2. The value for the highest dose of fast neutrons was ignored also in this figure. The D_0 (or D_{37}) values obtained are 52 rads with fast neutrons and 182 rads with gamma rays. Comparison of these two D_0 values gives an RBE value of 3.50 for 14.1 MeV fast neutrons as compared with ^{137}Cs gamma rays.

Postirradiation growth of hairs (*i. e.*, increase in number of hair cells) was also studied. Utilizing induced somatic pink mutations as markers, the average number of

Fig. 4. Average numbers of pink mutant events per hair at 11 or 12 days after irradiations with 14.1 MeV fast neutrons and ^{137}Cs gamma rays.

cells per hair in the buds at the time of irradiation could be estimated (see Ichikawa *et al.* 1969 for the method). From this estimation and from the average number of cells per hair observed in mature flowers of irradiated inflorescences, the postirradiation increase in the number of hair cells could be calculated (see Ichikawa and Sparrow 1967a for the procedure). The calculated values are plotted against dose in Fig. 3. The curves drawn in this figure give D_0 values of 56 rads and 191 rads with fast neutrons and gamma rays, respectively. Thus the RBE value for the effect of irradiation on hair growth by 14.1 MeV fast neutrons compared with ^{137}Cs gamma rays can be calculated to be 3.41.

An evident difference of efficiency was observed between the two different radiations also in inducing somatic mutations. The average numbers of pink mutant events per hair are plotted against dose on a log-log graph in Fig. 4. A pink mutant event is defined as a single pink cell or two or more contiguous pink cells which are considered to have been derived from a mutation induced in a single meristematic hair cell (Ichikawa and Sparrow 1968; Ichikawa *et al.* 1969). The data clearly show that the frequency of the pink mutant events increases with increase of dose in the range of lower doses, but then decreases with higher doses. Lines with a +1 slope are drawn in this log-log graph because the mutation frequency *vs.* dose relationship suggested a single-hit relationship in the range of lower doses. The two lines show that 5.05 times more pink mutant events were induced per unit dose with 14.1 MeV fast neutrons than with ^{137}Cs gamma rays.

On the postirradiation days 11 or 12, the terminal pink mutant events (those consid-

Fig. 5. Percentage of pink mutant cells in the terminal ten cells of hairs at 11 or 12 days after irradiations with 14.1 MeV fast neutrons and ^{137}Cs gamma rays.

Fig. 6. Average numbers of colorless events per hair at 11 or 12 days after irradiations with 14.1 MeV fast neutrons and ^{137}Cs gamma rays.

ered to have been derived from somatic mutations induced in the terminal cells of immature stamen hairs) found in hairs of normal length were mostly composed of more than ten cells. Thus it is considered that, on these days, the percentage of pink mutant cells in the cell population of the terminal ten cells (mostly those produced after irradiation) can be used as another measure of somatic mutation frequency. The calculated percentages are plotted against dose on a log-log graph in Fig. 5. Since the percentages for the two kinds of radiation reached peaks with lower doses compared with the average number of pink mutant events per hair shown in Fig. 4, the lines with a slope of +1 were drawn based on the data from only the two lower doses of fast neutrons and from only the lowest dose of gamma rays. Thus the RBE value from this figure is considered to be less reliable, of course, but the value obtained, 4.82, is close to the RBE value of 5.05 from Fig. 4.

Colorless cells were also induced by the radiation treatments. The average numbers of colorless events (defined as above for pink mutant events) per hair induced with fast neutrons and gamma rays are shown in Fig. 6 on a log-log graph. The lines drawn in this figure have a slope of more than one (+1.4), since the increase of the colorless events was not in direct proportion to dose but the events increased exponentially with dose. The RBE value obtained from this figure is 5.32, which is not far from the values of about 5 for pink mutation.

DISCUSSION

The relative biological efficiency (RBE) of 14.1 MeV fast neutrons as compared with ^{137}Cs gamma rays in the stamen hairs of *Tradescantia reflexa* KU 7 clone was about 3.4 to 4.0 for the loss of reproductive integrity (see Figs. 1, 2 and 3) and about 4.8 to 5.3 for the induction of somatic mutations (see Figs. 4, 5 and 6). These values are much lower than the 10 to 50 reported for higher plants with 14.1 or 14.7 MeV fast neutrons (Matsumura 1961, 1964, 1966; Matsumura and Nezu 1961; Fujii 1964a, 1964b; Smith 1967), or the 10 to 100 with the higher LET (linear energy transfer) 0.08 to 4.7 MeV fast neutrons (Neary *et al.* 1963; Davies and Bateman 1963; Fujii 1964b; Smith *et al.* 1964; Matsumura and Mabuchi 1965; Matsumura 1966; Smith 1967, 1969; Ahnström *et al.* 1969; Underbrink *et al.* 1970), or the 10 to 50 with other heavy particles (Matsumura *et al.* 1963; Fujii *et al.* 1966; Moutschen *et al.* 1968; Ikushima unpub.). Most of the earlier high RBE values (excepting those by Neary *et al.* 1963, Davies and Bateman 1963, Matsumura *et al.* 1963 and Underbrink *et al.* 1970) have been obtained from irradiation of dry or dormant seeds whose water contents usually fall between only 8 to 15 percent. It has been repeatedly demonstrated that X-ray or gamma-ray sensitivity is modified greatly by water content while neutron sensitivity is changed relatively slightly (Ehrenberg *et al.* 1952; Ehrenberg and Nybom 1954; Ehrenberg 1955; Ikushima 1968; Fujii 1969). Thus, at a low level of water content such as 8 to 15 percent seeds are much more resistant to X or gamma rays than at higher levels of water content, but water content does not affect the sensitivity of seeds to fast neutrons so much. Consequently, high RBE values are obtained by irradiation of dry seeds. The low RBE values obtained from the single meristematic (terminal) cells of actively growing *Tradescantia* stamen

hairs (containing a comparable amount of water with other organisms) confirms the concept that water content modifies RBE values. The present RBE values are more comparable to those from other organisms than to the values from dry plant seeds. Similar low or slightly higher RBE values have been obtained in *Tradescantia* microspores with 1.3, 2.5 and 14.1 MeV neutrons (Conger *et al.* 1958) and in growing *Vicia*, *Pisum*, *Helianthus*, *Raphanus* and *Arabidopsis* plants with 1 MeV neutrons (Donini *et al.* 1967). It has been also proved recently that low RBE values are obtained by preirradiation soaking of wheat (Ichikawa 1970), oat (Ikushima 1968) and *Arabidopsis* seeds (Fujii 1969) with 14.1 MeV neutrons.

The study by Davies and Bateman (1963) showed that the RBE of 0.65 MeV neutrons for inducing somatic mutations in *Tradescantia* stamen hairs was 17.5. They also demonstrated that the maximum RBE reached 40 when the two-hit component was excluded from the calculations. Neary *et al.* (1963) reported that the RBE of 0.7 and 3 MeV neutrons for various chromosomal aberrations averaged about 35 in *Tradescantia* microspores, but the value for only one-hit aberrations could be as high as 100. Thus it is expected that higher RBE values will result with X- or gamma-ray irradiation at lower dose rates (no change expected in neutron effects by changing neutron dose rates) as well as with irradiation of dried systems, since these conditions will produce fewer two-hit events. The marked difference in RBE values between the previous studies and the present study is considered to depend on difference in the LET of the neutrons, different X- or gamma-ray dose rates, and different dose ranges applied.

The higher RBE values obtained in the present study for the induction of somatic mutations than for the loss of reproductive integrity seem to indicate that fewer two-hit events were involved in the former than in the latter. Yet, an exponential increase of somatic mutation frequency with dose was observed in the case of the induction of colorless mutations, indicating the involvement of chromosome breaks (Fig. 6). In the range of gamma-ray doses applied in the present study, pink mutations increased linearly but soon reached a peak and dropped off (Fig. 4). This indicates that the dose range applied was too high to observe a similar exponential increase of pink mutation frequency. It is thus concluded that at least a part of colorless events were the result of chromosomal breaks. It is not as evident that a similar process was involved in the induction of pink mutations.

The D_0 value of 182 rads determined with ^{137}Cs gamma rays for the KU 7 clone of *T. reflexa*, a tetraploid species, is more comparable to those obtained earlier with the diploid clone, *Tradescantia* clone 02, than to those from other tetraploids. Davies (1963) reported 149 R as the D_0 for the stamen hairs of *Tradescantia* clone 02 treated with X rays at a dose rate of 108 R/min, and Ichikawa and Sparrow (1969) obtained a similar D_0 value of 154 R with 23.5 R/min ^{137}Cs gamma-ray irradiation of the same clone. The present D_0 value, which was obtained with gamma-ray irradiation at dose rates of 18 to 45 rads/min, is only about 1.2 times higher than these values. On the other hand, the present D_0 differs from the D_0 values of 360 R, 527 R and 675 R for *T. virginiana*, *T. rosea* and *T. crassifolia*, respectively, all of which are tetraploid species (Ichikawa and Sparrow 1967b). It should be noted, however, that these earlier values for tetraploids were obtained for less acute 16-hr exposures (0.10 to 0.78 R/min). In fact, the

D_0 of 270 R obtained by Nayar and Sparrow (1967) with the 16-hr irradiation of the clone 02 mentioned above is much higher than the D_0 s reported for the same clone treated at higher dose rates (see above). A similar dose-rate effect was also clearly observed in the stamen hairs of *T. blossfeldiana*, a twelve-ploid species. Namely, the D_0 for the 16-hr exposures was 1880 R (Ichikawa and Sparrow 1967a) while those for the more acute exposures were 720 R (Ichikawa and Sparrow 1969) or 767 R (Ichikawa 1968). It is thought that the difference in the D_0 values between species depends mainly on the different interphase chromosome volumes characterizing each species rather than the degree of polyploidy (clone 02, *T. virginiana* and *T. reflexa* have large ICVs; *T. rosea* and *T. crassifolia* have medium ICVs; *T. blossfeldiana* has a small ICV), as well as on the different dose rates. The relationship of radiosensitivity to the interphase chromosome volume in *Tradescantia* stamen hairs has been discussed elsewhere (Ichikawa and Sparrow 1967b).

SUMMARY

Inflorescences of *Tradescantia reflexa* (4x, 2n=24) KU 7 clone which is heterozygous for flower color (blue/pink, blue being dominant) were irradiated acutely with 37 to 141 rads of 14.1 MeV fast neutrons and with 144 to 362 rads of ^{137}Cs gamma rays. The stamen hairs of the irradiated and control flowers were examined daily for 19 days after irradiation.

At the time when the maximum radiation effects were observed, 1) the average number of cells per hair, 2) percentage of stunted hairs, 3) postirradiation growth of hairs, 4) pink mutant events per hair, 5) percentage of pink mutant cells, and 6) colorless events per hair were calculated for each dose and the effects of fast neutrons and gamma rays were compared. The determined RBEs of 14.1 MeV fast neutrons as compared with ^{137}Cs gamma rays for the above six end points were 1) 3.95, 2) 3.50, 3) 3.41, 4) 5.05, 5) 4.82, and 6) 5.32, respectively. These RBE values are much lower than those usually obtained from higher plants (mostly dry seeds) and are closer to those from other organisms. This point is discussed with respect to water content and dose-rate effects.

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