

# FRACTIONATED X-RAY DOSE AND CHROMOSOME ABERRATIONS IN BARLEY

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THE early experiments of Sax<sup>1,2</sup> which showed that the yield of chromosome aberrations was greater at higher intensities of irradiation and which were interpreted as indicating that restitution and reunion of broken ends take place within a very short time of their occurrence, pointed the need for studying the effect of dose fractionation on chromosome breakage. Experiments of this nature by a number of investigators with *Tradescantia* as the experimental material have given conflicting results, leading to two opposite interpretations—one supporting the theory of immediate restitution and reunion, the other consistent with the conclusion that the breaks may remain open for 12 hours or more.

In the present study, barley root tips were irradiated with an X-ray dose of 2,400 r given continuously or divided into two equal fractions separated by 2, 4, 6 and 8 hours. The continuous dose or the first fraction of it was given to the root tips of seeds which had been kept for germination for 15 hours. Observations on the division cycle in the root tips had been made earlier and it had been determined that the cells, in the 15 hours sample, exist in resting stage and do not emerge from this condition, i.e., enter prophase until about 9 hours later. In this way it was ensured that both the continuous dose and

its variously separated fractions were given to cells in the same stage. In order to find out whether the sensitivity of the chromosomes and other factors determining breakage yield vary during the course of the resting stage, the continuous dose was also given to 17, 19, 21 and 23 hours old samples, thus covering the entire length of the resting stage over which the two fractions were spread. All the fixations were made 13 hours after the beginning of the first exposure, with the exception of the above four samples which were fixed after shorter periods as shown in Table I. The root-tips irradiated with the continuous or the variously fractionated dosages were treated with a 0.2% aqueous solution of colchicine for a period of 5 hours immediately before fixation in order to arrest the cells at metaphase for scoring the aberrations. The irradiation was done at an intensity of 2,400 r per minute at a distance of 15 cm.; the operating voltage being 50 kV. The germinating seeds before and after the treatment were kept at a constant temperature of  $24 \pm 1^\circ \text{C}$ . in a Cenco incubator. The Feulgen squash method coupled with counterstaining in aceto-carmine was used for making the preparations.

The aberrations recorded as chromosome reunions were the dicentric chromosomes and the centric rings; those recorded as breaks,

TABLE I

Showing the frequencies of reunions and breaks due to (i) a 2,400 r continuous dose given at different periods of the resting stage, (ii) a single half-dose of 1,200 r, and (iii) the 2,400 r dose separated into two equal fractions

Interval between the fractions (hrs.)	Interval between fixation and First Exposure      Final exposure (hrs.)                      (hrs.)		Chromosome reunions per 100 cells	Chromosome breaks per 100 cells
0	13	13	$21.43 \pm 2.258$	$98.57 \pm 4.845$
0	11	11	$20.58 \pm 2.100$	$94.95 \pm 6.357$
0	9	9	$13.38 \pm 2.295$	$98.03 \pm 6.210$
0	7	7	$17.62 \pm 2.516$	$94.24 \pm 5.787$
0	5	5	$15.80 \pm 2.361$	$93.66 \pm 5.743$
0 (s.h.d.)	13	13	$4.65 \pm 0.830$	$32.88 \pm 2.22$
2	13	11	$6.15 \pm 1.039$	$33.91 \pm 2.441$
4	13	9	$11.42 \pm 1.420$	$50.53 \pm 3.003$
6	13	7	$14.67 \pm 1.694$	$69.47 \pm 3.686$
8	13	5	$14.93 \pm 1.590$	$98.09 \pm 4.127$

C.D.  
6.42

C.D.  
16.11

the acentric fragments and the minutes. The chromatid aberrations were not taken into consideration because relatively few of them were produced with any of the treatments. Several hundred cells, without any selection, were analysed in each case for the scoring of the aberrations.

The observations in respect of each of the treatments are summarised in Table I. These show that when the radiation dose is given in two fractions with an interval of 2 hours between them, the frequencies of both types of aberrations are considerably reduced compared with those resulting from the continuous dose. When the interval between the fractions is extended to 4 hours, a reduction which however is less pronounced, can still be observed. As the gap between the fractions is still further increased, the aberrations frequency approaches more closely the breakage yield due to the continuous dose. The data thus show that the drop in the number of chromosome aberrations when the radiation dose is spread over two fractions with a relatively short interval between them is followed by a recovery which appears to reach a maximum for the reunion when the interval extends to 8 hours. Further, the aberration frequencies due to the single half dose of 1,200 r, also indicated in Table I, make it clear that the yield, on fractionation, drops below twice this value and rises above it, following the process of reduction and recovery.

A correct interpretation of results from investigations designed to study the dose fractionation effect, is rendered difficult because of the fact that radiation sensitivity of the chromosomes, as also the conditions determining restitution and reunion of breaks, are known to vary during the course of the nuclear cycle.<sup>3</sup> The possibility that the changed frequency of the aberrations following fractionation of the dose might be due to the altered sensitivity or the different conditions for restitution and reunion in the two samples, has therefore to be taken into consideration. The observations in Table I show that neither the frequency of the reunions nor that of the chromosome breaks differ significantly when the continuous dose is given to cells at different stages of the resting condition, except in the case of the sample fixed 9 hours after irradiation, in which the frequency of reunions is slightly reduced. It is obvious that neither the extent of this reduction, nor the stage at which it occurs, suggest a possible change in the reduction-recovery trend of the dose fractionation data.

In view of these considerations, it may be supposed that the differences in the frequencies of the reunions and breaks due to the continuous and the fractionated dosages are primarily a function of dose fractionation.

The initial drop following fractionation of the dose as described here is in full agreement with the corresponding observations of Sax and Luippold,<sup>4</sup> Lane<sup>5</sup> and others<sup>6,7</sup> on *Tradescantia*. Sax interpreted this drop as due to a possible property of the broken ends to reunite, reconstitute or heal within an hour, usually within a few minutes of the breakage and therefore failing to take part in reunions with those produced by the second fraction given after an interval of more than one hour.

Experimental observations opposed to this interpretation of the decline in the breakage yield were first described by Lane (*loc. cit.*). These showed that the initial drop was followed by a recovery as the interval between the fractions was extended beyond 4 hours. This recovery which is obviously not consistent with the theory of rapid reunion and restitution was not apparent in the experiments of Sax and a number of other authors. It has been suggested that the different conditions of experiments may be responsible for the contradictory results.

The present study in barley indicates a recovery in the frequency of aberrations similar to that reported for *Tradescantia*, although the two differ in their timings. The extent of this recovery suggests that the breaks due to first fraction remain open for reunion with those produced by the second, several hours later. Similarly the extent of the drop preceding the recovery is not fully explained by the hypothesis of rapid reunion and restitution. An alternative explanation of the dose fractionation results, however, is not very evident. Lane has suggested that the drop and recovery are due to a physiological effect of the radiation treatment, which makes the chromosomes temporarily less sensitive to subsequent irradiation.

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