FREQUENCY OF CHROMOSOME ABERRATIONS PRODUCED BY FRACTIONAL DOSES OF X-RAYS IN TRADESCANTIA[1]

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THEORIES based on results from fractional dose and time-intensity X-ray experiments using Tradescantia inflorescences have become important cornerstones for interpretations of many irradiation experiments. The major evidence has been amassed and theoretical considerations developed by SAX (1939, 1940 and 1941), FABERGÉ (1940), LEA and CATCHESIDE (1942), CATCHESIDE, LEA and THODAY (1946a and b) and CATCHESIDE (1948). According to these workers most chromosome breaks in irradiated Tradescantia restitute or undergo reunion soon after their production and such breaks do not reopen. Because of this rapid reunion or restitution the frequency of two-hit aberrations (i.e., chromatid interchanges, dicentric chromosomes and centric rings) is higher at high intensity than at low intensity. The experiments of SAX on the effects of fractional X-ray dosage have shown that after about one hour from the time of irradiation breakage-ends no longer participate in reunions.

Recently LANE (1951) has questioned most of these findings and their resulting interpretations and has rejected the theory of rapid reunion. LANE's data on reunions presented in his graph 5 show that the highest frequency of breaks was produced by a single dose of 360 r. Thereafter a progressive decrease in the frequency of aberrations occurred when two 180 r doses were separated by 1, 2 and 4 hours.

Reunion frequency was higher when dose-fractions of 180 r each were separated by 6 or 8 hours than when the interval was 4 hours. When the time interval was 8 hours the frequency of reunions was almost as high as that obtained with a single dose of 360 r. The apparent sharp rise in reunions in the 8-hour fractional material led LANE to reject the theory of rapid reunion of X-ray breaks. In his view chromosome breaks produced in the resting nucleus by X-rays may remain open for several or many hours, restitution and reunion being delayed until the beginning of prophase.

The low reunion frequency in the 4-hour fractionation group is attributed to physiological effects of the first radiation fraction on the chromosomes. The chromosomes respond to the radiation by becoming less liable to further breakage by X-rays administered at this time. This newly developed resistance to breakage is highest after about 4 hours but has almost disappeared by 8 hours.

[1] Most of this work was carried out while both authors were at University of California, Berkeley, California. A part of the work was done at the Brookhaven National Laboratory under auspices of the U. S. Atomic Energy Commission and a part was done at the University of Saskatchewan.

LANE attributes the high reunion frequency observed when dose-fractions are 8 hours apart to persistence of open breaks produced by the first dose fraction until after the second group of breaks has appeared. LANE concluded that the chances of reunion are thus very similar in cells given a single dose of 360 r and of cells given two doses of 180 r separated by an interval of 8 hours.

SAX and LUIPPOLD (1952) have repeated LANE's 1951 experiments with *Tradescantia paludosa*. They used a continuous 360 r dose and three fractionations, (2 doses of 180 r in each) the interval between fractions being 4, 8 and 12 hours. A single dose of 180 r was used to establish a base line. The number of aberrations was higher from single than from divided 360 r doses in these experiments. The frequencies of reunions in the 4, 8 and 12 hour fractionated sets were very similar and were approximately equal to those produced by a single dose of 180 r multiplied by 2. These authors failed, therefore, to confirm LANE's essential findings, namely: (1) very low reunion frequency in the 4-hour fractionated-dose material and (2) high reunion frequency in the 8-hour fractionation group. LANE (1952) raised the following objections when he questioned the experimental procedure of SAX and LUIPPOLD: Failure to control temperature after irradiation; use of different experimental material; different X-ray intensity; and different time period between irradiation and fixation.

Firm establishment of results from fractionated X-ray dose experiments is sufficiently important to radiobiological theory that experiments were performed to repeat LANE's 1951 experiment. In these experiments a particular effort was expended to overcome the objections raised by LANE concerning SAX and LUIPPOLD's procedure. The experiments here presented had three general objectives: (1) to repeat LANE's 1951 experiment as closely as was possible. (2) To include two additional irradiation periods of 12 and 24 hours along with single doses of 180 r and 360 r at all fractionation periods to be certain that nuclear sensitivity was constant. (3) To examine cell samples of adequate size and with repetitions so the data would not be seriously questioned because of possible sampling or experimental errors.

MATERIALS AND METHODS

Material used in the study was *Tradescantia paludosa* (clone 5 of SAX). The plants were potted in soil and only young inflorescences (excised from plant) were used. All buds within each experiment were fixed at the same time in ethanol acetic acid (3:1). Samples, depending on the treatment, were irradiated at zero time (3 days before fixation); and at 4, 8, 12 and 24 hours later. These correspond respectively to a post-irradiation time of 72, 68, 64, 60 and 48 hours. Chromosomes were stained with carmine and examined at the first microspore division from permanent slides. Only clear metaphase figures were scored.

The X-radiation, administered with a General Electric Coolidge tube operated at 100 KVP and 3 milliamps was filtered through 1 mm of aluminum. The distance from the target to the inflorescence was 31 cm in experiments

TABLE 1

The effect of fractional dosage on the frequency of chromosome aberrations.

Dose	Time of irradiation (hours)		Exp. no.	Chromosomes scored	Dicentrics and centric rings		Interstitial deletions		Chromosome breaks	
					Percent	χ^2	Percent	χ^2	Percent	χ^2
"Continuous" 360 r	0		1	5502	5.72	0.11	7.67	6.67	7.42	1.80
	0		2	5826	5.66	0.29	6.73	1.16	6.62	0.82
	0		3	1242	5.80	0.003	6.52	0.60	5.56	3.42
	8		1	3288	5.60	0.32	6.90	0.19	8.70	14.67
	12		2	2760	6.59	2.73	6.96	0.09	6.52	0.69
	24		2	930	5.05	0.97	7.74	0.53	5.81	1.72
	24		3	1464	6.56	1.31	5.94	2.79	5.12	6.96
TOTALS				21,012		5.74		12.02		30.09
Grand means with standard errors.					5.83 ± 0.17		7.10 ± 0.18		6.94 ± 0.41	
P values at 6 D.F.					.50–.30		.10–.05		<.001	
	1st	2nd								
Fractional doses 180 r—180 r	0	4	1	2016	4.71	0.82	4.66	3.70	6.60	4.68
	0	4	2	4806	4.60	1.15	5.35	0.96	5.14	1.46
	0	8	1	3258	4.42	0.12	7.15	12.35	6.05	0.34
	0	8	2	2310	4.42	0.10	5.89	0.21	4.24	7.12
	0	12	1	3936	3.89	1.53	6.63	6.21	7.06	1.62
	0	12	2	4356	4.34	0.02	5.46	0.39	5.72	0.40
	0	24	2	3228	3.81	1.76	4.34	10.30	3.87	16.37
TOTALS				23,910		5.51		34.12		21.99
Grand means with standard errors.					4.30 ± 0.13		5.68 ± 0.34		5.55 ± 0.34	
P at 6 D.F.					.50–.30		<.001		<.001	
Single 180 r	0		3	3084	2.20	0.75	2.89	0.07	2.46	1.51
	4		3	2724	2.39	0.04	3.01	0.41	3.08	0.58
	8		3	4608	2.56	0.23	2.82	0.005	2.69	0.34
	12		3	3188	2.73	1.02	2.70	0.13	2.98	0.32
	24		3	1626	2.15	0.58	2.71	0.69	3.26	1.02
TOTALS				15,230		2.63		1.31		3.79
Grand means with standard errors	(2 × for base line values)				2.45 ± 0.13		2.80 ± 0.14		2.84 ± 0.14	
P at 4 D.F.					.70–.50		.90–.80		.50–.30	

1 and 2, and 30 cm in experiment 3. At these distances the intensity of irradiation as measured with a Victoreen dosimeter was 51 r/minute in experiments 1 and 2, and 59 r/minute in experiment 3. The Victoreen instrument was also used to measure the total irradiation received by the inflorescences. Readings recorded were within the 5% error of the dosimeter and verified the calculated doses which are given in the data.

The " continuous " doses of 360 r as implied by the quotation marks were not actually continuous. In experiments 1 and 3, doses were given in two 180 r fractions separated by one minute. In experiment 2 the doses were separated by 2 minutes. This time interval permitted recording the actual doses received although such a procedure was probably unnecessary because calculated doses were well within the 5% accuracy of Victoreen readings. These short fractionations served to lower slightly the number of breaks available for reunion for only a minute or two during the treatment. Any difference in the frequency of two-hit aberrations between these short fractionations and a continuous dose probably would go undetected with even large sample numbers. For simplicity of presentation these short fractionations are referred to as " continuous " doses of 360 r.

The temperatures during irradiation were $23 \pm 0.4°C$, $22.5 \pm 0.5°C$ and $23.7 \pm 0.7°C$ for experiments 1, 2 and 3 respectively. The 0.7 to 1.2° higher temperatures in experiment 3, which would be expected to yield slightly fewer observed breaks (SAX 1947 and CATCHESIDE 1948), were compensated for by a higher dose intensity of 59 r/minute. The higher intensity should bring up the yield of two-hit aberrations to those of experiments 1 and 2. As a result dicentrics and rings should be homogeneous in all experiments. Probably one-hit aberrations would not be homogeneous and would occur at a lower frequency in experiment 3 than in experiments 1 and 2.

After irradiation, inflorescences were transferred to a chamber. The average daily illumination in experiment 1 was 11.3 hours and 12.2 hours in experiments 2 and 3 which approximated natural light conditions in March and April 1952. Until the material was fixed temperatures were maintained at $23.6 \pm 1.6°C$, $22.2 \pm 2.1°C$ and $22.0 \pm 2.0°C$ in experiments 1, 2 and 3 respectively.

RESULTS

The data from these three groups of treatments are given in table 1. The first group of data is from inflorescences exposed to " continuous " 360 r doses. The second group in table 1 was obtained from inflorescences given two doses of 180 r spaced by 4, 8, 12 and 24 hours. The final group was from material irradiated with single doses of 180 r at 0, 4, 8, 12 and 24 hours. The base line of SAX (1941) was obtained from these single 180 r doses by multiplying the frequency of each type of chromosome aberration by two. The three general categories of chromosome aberrations tabulated in table 1 are: dicentric chromosomes and centric rings (reunions), interstitial deletions (minutes) and chromosome breaks. Chromatid aberrations were so infrequent that they were ignored in the scoring.

When tested by the chi square method for homogeneity within groups, dicentrics and centric rings were homogeneous in each case as indicated by P values (see CATCHESIDE *et al.* (1946a) for method of calculation) in table 1. The vertical lines in figure 1 are confidence limits of means at the 95% level. They were derived from SNEDECOR (1946) in the " values of t table " and are presented for dicentrics and centric rings. The grand means and their confidence limits for centric reunions, chromosome breaks and interstitial deletions are separately recorded in table 2. As indicated by the means and confidence limits of .95 found in figure 1 and table 2, the " continuous " 360 r group was

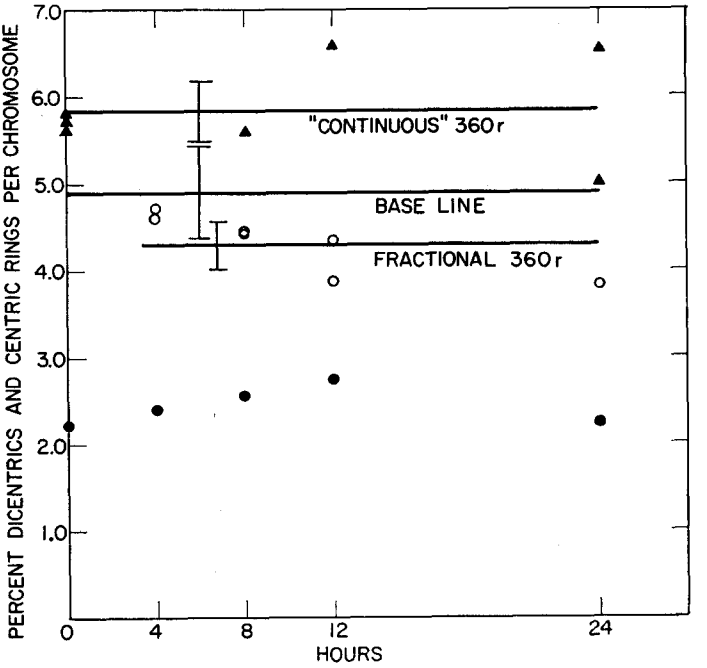

FIGURE 1.—The horizontal lines represent grand means of each treatment group. The base line consists of the grand mean of single 180 r samples multiplied by two. The vertical lines describe confidence limits of each mean at the 95% level. Individual sample points are: ▲, "continuous" 360 r; ○, fractional 360 r (180 r–180 r); and ●, single 180 r.

significantly higher than both fractionated 360 r and base line (2 × single 180 r) groups. The means of fractionated and base line groups did not differ significantly from each other, lying within the 95% limits.

From figure 1 it is apparent that the parameter of the base line estimated from these data could by chance be above or between the various points of the fractionated doses. Because of the overlap of fractionated 360 r and base line means and the homogeneity within both, it is concluded that none of the time interval frequencies were significantly different from the base line. However with increased time intervals between dose-fractions there was a downward trend of the frequency of dicentrics and centric rings (figure 1). Whether

the trend is real or due to chance alone cannot be determined with the chi square test.

Within " continuous " 360 r doses and single 180 r doses interstitial deletions were homogeneous (table 1). Fractionated 360 r doses however had excessive variation. The major chi square contribution came from the two samples, where time between doses was 8 and 24 hours. The second repeat with the 8-hour interval deviated little from the mean which tends to discount any significant increase of interstitial deletions. With the 24-hour split dose however it is not clear whether the sample is significantly lower or if the deviation is due to sampling error.

Grand means of interstitial deletions in " continuous " doses of 360 r are significantly higher ($P < .01$) than those of the fractionated and base line groups.

TABLE 2

The frequency of chromosome aberrations in Tradescantia microspores following "continuous" 360 r, fractional 360 r and single 180 r (× 2) doses of X-rays. Grand means and their confidence limits at the 95% level are expressed in percent of total chromosomes.

	"Continuous" 360 r	Fractional 360 r	Base line (2 × aberrations of single 180 r)
Dicentrics and centric rings	5.83 (5.49–6.17)	4.30 (4.04–4.56)	4.90 (4.38–5.42)
Interstitial deletions	7.10 (6.74–7.46)	5.68 (5.00–6.36)	5.61 (5.05–6.17)
Chromosome breaks	6.94 (6.12–7.76)	5.55 (4.87–6.23)	5.67 (5.11–6.23)

The aberrations scored as chromosome breaks were acentric fragments and acentric rings (× 2). Excluded from chromosome break counts were acentric fragments which came from chromosomes observed as dicentrics and centric rings. The assumption was made that each pair of fragments from these reunion chromosomes always united to form single acentric fragments.

A within-group test for homogeneity in table 1 showed that " continuous " and fractionated 360 r doses were not homogeneous for chromosome breaks, while the single 180 r group was homogeneous. This excess variation may have resulted from sampling errors or more likely scoring errors. Some variation could be due to the fact that samples were irradiated at different temperatures. The samples in experiment 3 were irradiated at higher temperatures than 1 or 2 and so, as is the case, would be expected to have a lower frequency of one hit chromosome breaks.

As seen from table 2 there was no significant difference between means of chromosome breaks in the three groups at the 95% level. The high mean of the " continuous " dosage group even though not significantly higher, could be explained by the partial two-hit component in the chromosome break class.

DISCUSSION

The data presented completely verify the findings and conclusions of SAX (1939, 1940 and 1941) and SAX and LUIPPOLD (1952). In Tradescantia most chromosome breaks, measured by the frequency of dicentrics and centric rings, or other two-hit aberrations, restitute or undergo reunion soon after they are produced (see also CATCHESIDE 1948). In Tradescantia microspores once reunion or restitution has occurred the breakage ends involved are permanently removed from the pool of open breaks in the nucleus still available for rejoining. The evidence does not support the contention of LANE (1951) that restitution and reunion are delayed for several or many hours.

LANE's hypothesis that when a dose of 360 r is divided into two equal fractions the first fraction produces temporary physiological effects on chromosomes that render them less liable than before to radiation breakage is not supported. According to LANE these effects are maximal near 4 hours after the first fraction and are greatly reduced by 8 hours. The recovery at 8 hours as shown by an increased frequency of centric reunions did not occur in our experiments nor in those of SAX and LUIPPOLD (1952). In our material reunion frequencies were very similar in all lots given fractional X-ray treatments with a trend for a slight reduction in reunion frequency as the time between fractions was increased from 4 to 24 hours. However, the differences using the chi square test were too small to be statistically significant.

Our base line for centric reunions lies slightly above the means for samples receiving fractional doses. Somewhat similar results were reported by SAX (1939 and 1940) and by LANE (1951), where his fractions were 2, 4 or 6 hours apart. In other reported experiments the base line has been slightly below the values from split-dose experiments (SAX 1941 and SAX and LUIPPOLD 1952). Chance alone may determine, in a particular experiment, whether the base line will be above or below the value of the fractional-treatment group. It seems evident that when the dose fractions are 4 to 24 hours apart the reunion frequencies are not significantly different from those of the base line. It thus appears that breakage ends produced by the first fraction of a dose do not participate in reunions with breakage ends produced 4 to 24 hours later. All previous investigations were in agreement on the lack of participation of breakage ends from the first fraction with 1 to 4 hours between.

SAX (1939 and 1941) and CATCHESIDE *et al.* (1946b) found chromatid breaks independent of irradiation intensity. In this study (table 2) chromosome breaks, even with a minor two-hit component, were homogeneous. This homogeneity supports the theory that one-hit breaks are independent of irradiation intensity or dose fractionation.

According to SAX and ENZMANN (1939), CATCHESIDE *et al.* (1946b), SAX (1947) and CATCHESIDE (1948) more breaks are produced at low temperatures during irradiation than at high temperatures. In part the chromosome break frequencies recorded in table 1 were lower at the higher temperatures during irradiation. Because dose intensity compensation was employed

to counter the temperature effect two-hit aberrations were essentially the same. Increased breakage caused by low temperature was found due to higher oxygen tension by GILES and BEATTY (1950). GILES, BEATTY and RILEY (1951) found that in the absence of oxygen fewer breaks were induced at low temperatures and more at higher temperatures. LANE's concern (1951 and 1952) about exactly controlled temperature after irradiation (with little emphasis on temperature during irradiation) seems partially unjustified. The effects of temperature shock shown by SAX (1937), LACOUR (1949), and CALDECOTT and SMITH (1952) at extreme high and low temperatures should be avoided. However between irradiation and fixation at normal room temperatures, nuclear sensitivity for *Tradescantia paludosa* microspores is constant for the period 2–5 days before metaphase of the spore nucleus according to SAX (1941), GILES and RILEY (1949) and evidence presented here. Slight differences in the rate of development will have little if any effect on break frequencies when the material is fixed on the third or fourth day after the irradiation treatments, thereby making exactly controlled temperatures after irradiation unnecessary.

SUMMARY

When Tradescantia microspores were irradiated with 360 r of X-rays, the frequency of centric reunions was consistently higher with a single "continuous" dose than with two 180 r doses separated by 4, 8, 12 and 24 hours. In the fractional dose series there was no significant difference in reunion frequency with increasing time intervals between fractions. This fractional series was not significantly different from the base line. The frequency of one-hit chromosome breaks appeared to be independent of dosage fractionation. These findings confirm the observations of SAX, CATCHESIDE and other workers. Conversely the evidence does not support the findings of LANE where reunions and chromosome breaks were low when two 180 r doses were separated by 4 hours and were high when separated by 8 hours. The majority of previous investigations and the data presented here agree with the theory that Tradescantia chromosome breaks restitute or undergo reunion soon after their production. It is also apparent that restituted breaks rarely if ever reopen.

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