AN EXPERIMENT ON CHROMOSOME FRAGMENTATION IN *TRADESCANTIA* BY X-RAYS

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(With Two Text-figures)

Introduction

That X-rays cause structural chromosome changes has been known for a long time. More recently, attempts have been made to correlate this approach to the subject with the results of purely genetic methods. These genetic methods of studying artificial mutation, though simple and objective, are laborious. Most conclusions are based on the detection of a collective class of lethal mutations, though it is true that none of these conclusions are contradicted by what meagre data are available on changes at single loci. It is now realized that at least a considerable proportion of such lethals are associated, or even perhaps identical with structural chromosome changes. Hence the attempts to verify and extend the results of genetic work by less tedious cytological means. The present paper is a contribution to this subject.

Ideally, one would like to identify all structural changes in a cell at any desired time after irradiation. No one method of doing this exists. In the salivary glands of *Drosophila*, it is possible to identify most structural changes, but only after the chromosomes concerned have passed through numerous cell generations, during which many structures are selectively eliminated; breaks in which both former partners have rejoined cannot be scored. In the microspore divisions of plants, on the other hand, chromosomes can be observed within a few hours after treatment, without intervening cell divisions; but even in the best material only some of the most obvious structural changes can be identified with certainty. The pollen grain division method is also by far the least laborious, and is the one used in the present work.

The previous work which most closely resembles that about to be presented is due to Sax (1938, 1939) and to Sax & Enzmann (1939). Sax was interested in the same problems, and used similar material; but his method of scoring was quite different, and he used smaller dosages. It is therefore difficult to make direct comparisons with his data. Refer-

ences to older work of a similar kind will be found in those papers, and in the excellent review by Goodspeed & Uber (1939).

An important work on induced rearrangements studied in salivary glands is that of Bauer *et al.* (1938); a full bibliography is given there. Theories on the action of radiation from the genetic point of view are fully reviewed and discussed by Timoféeff-Ressovsky *et al.* (1935) and Stubbe (1938).

Some preliminary trials were made in collaboration with Dr K. Mather in 1935 (Mather, 1937). At that time no dosimeter was available, and no attempt was made to arrange the experiments so as to get a valid estimate of error. No conclusive results were obtained, but several technical difficulties were solved. It is a pleasure to express my indebtedness to Mr G. R. Ward, who was responsible for inventing the dosimeter used.

General plan of the experiment

Factors thought to be important for the interpretation of X-ray action on chromosomes are the following:

(1) the nature of the increase of effect with increasing dosage;
(2) the effect of the same ionization dose at different wave-lengths;
(3) the effect of fractionating, or of diluting the dosage in time;
(4) the effect of temperature at the time of irradiation.

To these may be added the effect of varying the physiological condition of the organism when treated, of increasing X-ray absorption in the tissues by heavy elements and similar variables. Factors 1, 3 and 4 are the subject of this paper. Factor 2, though important, was not included owing to technical difficulties and lack of equipment.

The general plan of the experiment was to give three dosages proportional to the numbers 1, 2 and 3 each, either continuously during 18 min. or in six exposures of 3 min. during 3 hr. (a 10× fractionation); each combination of dose and fractionation was given at a temperature of 15 and 30° C. Thus there are altogether twelve treatments. Fixation of the material was done 70 hr. (± 10 min.) after treatment. The list of treatments will be found in Table I.

The dosages used were those found to give conveniently analysable results in previous trials, though in the light of this experiment they are perhaps a little high. Not to complicate results unduly, it was thought best to keep the maximum time during which treatment is given small in relation to the period of one cell cycle. About 10 days elapse between meiosis and microspore division with this material, and it was judged

TABLE I

Experimental data

Dose	Continuous or fractionated		Number of chromosome fragments in cell																							Slide totals	Treatment totals
			5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	23	23	24	25	26	27		
15° C.	3	A	.	.	.	.	.	.	1	.	.	2	7	5	4	1	3	3	2	.	1	1	.	.	.	516	1578
			.	.	.	.	.	.	1	1	3	4	3	4	1	5	3	2	2	1	.	.	.	.	.	495	
			.	.	.	.	.	.	1	1	.	.	3	1	2	4	7	4	1	3	.	1	.	1	1	567	
		B	.	2	4	9	7	6	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	257	1065
			.	.	.	2	4	7	5	4	4	1	2	.	.	.	1	.	.	.	.	.	.	.	.	340	
			.	.	.	.	.	.	2	2	3	6	2	7	2	2	.	2	.	.	1	1	.	.	.	468	
	2	C	.	.	1	3	3	2	5	11	.	3	1	1	.	.	.	.	.	.	.	.	.	.	.	338	928
			.	1	5	7	2	6	2	4	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	285	
			.	2	4	1	6	2	5	5	4	.	.	1	.	.	.	.	.	.	.	.	.	.	.	305	
		D	.	.	4	5	6	9	3	1	.	.	.	.	1	1	.	.	.	.	.	.	.	.	.	292	791
			.	12	7	8	2	.	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	218	
			.	.	7	5	6	6	3	1	1	.	.	.	.	.	.	1	.	.	.	.	.	.	.	281	
	1	E	.	9	11	5	3	1	.	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	223	650
			.	9	7	5	6	2	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	229	
			.	16	10	4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	198	
		F	.	20	8	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	192	598
			.	18	7	5	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	197	
			.	12	11	4	2	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	209	
30° C.	3	G	.	.	1	3	2	3	5	6	1	6	2	.	1	.	.	.	.	.	.	.	.	.	.	350	1179
			.	.	.	.	2	1	4	6	5	1	4	4	.	2	.	1	.	.	.	.	.	.	.	403	
			.	.	.	.	.	1	2	8	5	1	1	6	3	1	1	1	.	.	.	.	.	.	.	426	
		H	.	4	4	8	5	4	3	.	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	261	704
			.	6	11	10	2	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	222	
			1	5	13	8	1	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	221	
	2	I	.	.	6	4	5	4	4	1	3	.	3	.	.	.	.	.	.	.	.	.	.	.	.	299	857
			.	1	5	8	5	5	2	3	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	274	
			.	2	4	6	4	4	5	2	2	.	1	.	.	.	.	.	.	.	.	.	.	.	.	284	
		J	1	1	21	5	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	216	652
			1	12	13	1	1	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	205	
			.	7	9	6	5	2	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	231	
	1	K	.	12	10	6	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	208	658
			.	17	7	3	1	.	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	206	
			1	5	8	3	7	4	1	.	.	.	1	.	.	.	.	.	.	.	.	.	.	.	.	244	
		L	1	13	11	3	1	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	203	586
			.	20	8	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	192	
			.	21	7	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	191	
			5	227	224	143	92	73	61	57	35	26	32	30	14	16	15	14	5	4	2	3	0	1	1	10,246	

that a 3 hr. period was small enough in relation to 10 days, and that no great changes would occur in the resting stage during a 3 hr. interval. In genetical work, very much greater fractionations have sometimes been used (e.g. $\times 1000$). The temperature difference was the greatest that could conveniently be obtained with the apparatus available; it was not thought safe to subject *Tradescantia* to more than 30° C.

No attempts appear to have been made in the past to arrange experiments so as to get a valid estimate of error, and no guidance was available as to the sources of the discrepancies in such work. Methods of experimental design due to Prof. R. A. Fisher were used by the writer; their adaptation to this new problem was made difficult by absence of previous experience, and it is not claimed that the best use was made of them.

One obvious procedure would have been to repeat the whole experiment a number of times, and to treat the replications like the "blocks" of a standard agricultural trial; but this would have been very tedious with fractionation of dosage as one of the variables. The plan was therefore adopted of trying to make the measurement of dosages, the timing, and the temperatures physically so precise as to render any replication of them superfluous. Admittedly, by proceeding in this way, one of the advantages of modern experimental design is deliberately discarded.

The three slides in each treatment were regarded as replications for the purpose of the analysis; they adequately sample variations in sensitivity of the material, and variations of dosage due to scatter and to the slight differences in distance from the tube. But an experimental error derived from these replicates is only valid if discrepancies in measurement of tube output and temperature are negligible in comparison. For this reason much care was given to the physical measurements, and they will be described in some detail.

Material and cytological methods

A clone of *Tradescantia bracteata* having $n=6$ chromosomes and no natural fragments was used, the plants being grown in pots in a cool greenhouse. The sixty inflorescences needed were cut off immediately before the experiment. For each of the twelve treatments five inflorescences were used; after irradiation they were kept in water at room temperature ($\pm 20°$ C.) and smeared 70 hr. (± 10 min.) after irradiation. It is known that 70 hr. before pollen grain division metaphase the chromosomes behave mainly as though "single" with respect to X-rays (Riley, 1936;

Mather, 1937). One anther was examined in aceto-carmine, and the remaining five smeared and fixed in 2BE (La Cour, 1937); gentian violet stain was used in the standard manner. To make the detection of small fragments easy, a strong and sharply contrasted effect was aimed at. About 100 slides were made.

A 2 mm. oil-immersion objective of N.A. 1·32 (Leitz) was used with ×10 eyepieces, giving a magnification of ×920. The method of scoring was simply to count the number of chromosome bodies in the cell at metaphase; it is the writer's opinion that only such a method is sufficiently objective for quantitative work. At first sight it might seem that much information could be obtained by recording obvious translocations, multiple attachment chromosomes, etc. But even in the best material such observations are very subjective. While in numerous instances every observer would agree, there is no standard by which to define the obvious. It is confidently believed that with the method adopted, the work could be repeated by even an untrained observer with essentially identical results.

The possibility of bias in the selection of cells for observation is an important one, and appears to have received but little attention. It is of course impossible to record every metaphase in a given slide area. Even in the best preparations some plates are not as well fixed as others, or badly orientated, or injured in smearing. If only the number of chromosome pieces is scored, the possibility of bias in selection is diminished, though not excluded. As a further safeguard, the following precaution was taken: the inscription on each slide had a paper label glued over it, and a set of random numbers assigned to the labels. By means of the number and a key each slide could be identified as belonging to some one of twelve groups corresponding to the twelve treatments but not to which particular treatment. Thus, during the cytological work, it was never known which particular treatment was being examined. Slides were first selected for satisfactory number of divisions, fixation and stain; it was then found that three slides only were available in one of the treatments. So as not to complicate statistical analysis, three slides were used in all treatments.

Drawings were made of thirty cells from each slide, and the fragments later counted on the drawings, thus making 1080 cells for the whole experiment. The number of cells available was of course much greater than thirty; the first thirty suitable cells coming into the field of the oil-immersion lens were used. Altogether, 10,246 chromosome pieces were recorded.

Temperature control

The problem of keeping an inflorescence of *Tradescantia* at a known constant temperature during X-ray treatment would at first sight appear simple. In actual fact much time had to be spent before this was satisfactorily done (Cullen & Fabergé, 1939).

An air thermostat was used, containing about 60 litres of air rapidly circulated in a closed path by a centrifugal fan. The whole apparatus was enclosed in a wooden box lagged with 7 cm. of sawdust, except for a cellophane window through which X-rays reached the plant chamber. The air current passed successively over: (*a*) the electric heating wires, (*b*) a grid of copper tubes through which cold brine circulated, (*c*) the thermo-regulator, (*d*) the plant chamber. The latter is a space $40 \times 20 \times 20$ cm. with the inflorescence 15 cm. from the cellophane window. No brine was of course pumped through the cooler for working above room temperature. The thermo-regulator was specially designed to have a very low lag; it consisted of 10 metres of thin-walled glass tubing about 1 mm. in diameter, containing about 10 c.c. of toluene. This capillary tubing was bent to form a grid in a space $10 \times 8 \times 12$ cm., through which the whole air stream passed. The hunting period of the thermostat was of about 17 sec., and no departures from the desired temperature could be observed on a thermometer graduated in 0·1° C.

By thermocouple measurements it was found that *Tradescantia* inflorescences placed directly in the air stream never attained thermostat temperature owing to evaporation; discrepancies as great as 2 or 3° C. occurred despite attempts to saturate the atmosphere. The difficulty was overcome by putting each inflorescence in a small oiled silk bag moistened inside. It was impractical to verify the temperature of the buds by thermocouples during the actual experiment. But separate determinations showed that under the conditions above described, inflorescences never took longer than 18 min. to be heated through a temperature interval of 10° C., and to be within 0·1° C. of the thermostat temperature. The routine was adopted of leaving the material in the thermostat for 30 min. before starting treatment.

It can be asserted with some confidence that the real temperature of the material was within 0·1° C. of that stated throughout the treatment period.

X-raying technique

The X-ray source available was a Müller hot-cathode (Coolidge) water-cooled 6 kW. glass tube with a tungsten target. Current was

supplied by a transformer adjusted to give 72 kV. peak, with a single wave electronic rectifier, and without any smoothing devices. Although that voltage is sufficient to excite the K spectral lines of tungsten, it is likely that the bulk of the radiation is white, since voltages above the critical are only applied during short periods of the cycle. The shortest wave-length with 72 kV. is 0·165 A.; then there is probably a peak at about 0·2 A., after which the energy tails off as wave-length increases. No filter was used, although the glass tube, the paper walls of the ionization chamber and oiled silk enclosing the buds probably suppressed most of the very soft rays. X-radiation of about that quality is often used for producing mutations.

A source of discrepancy often neglected in biological work is the simple fact that X-ray intensity is inversely proportional to the square of the distance from the target. In the present experiment, this distance was 30 cm., the material being packed in a 1 cm. layer between threads stretched on a wooden frame. Even so, the difference in intensity between the top and bottom of that 1 cm. layer is of about 6 %. It is true that the anthers themselves would tend to be in a thinner layer; on the other hand scatter and absorption introduce other discrepancies which are difficult to assess. These considerations make it plain that quite apart from variations in sensitivity of the material, experimental error must be based on variations between buds.

The dosimeter used will be described in a separate paper (Ward & Fabergé), where the conditions under which it gives accurate results will be discussed. It is sufficient to indicate here its general principle, and to say that empirical calibration has shown it to possess an accuracy ample for the present purpose.

The ionization chamber is of 20 cm.^{3} capacity with graphited paper walls and guard rings to prevent leakage. It is permanently fixed between the tube and the material at 7·5 cm. from the target, and intercepts all that part of the beam used for treatment. An electrical integrator, followed by an amplifier and magnetic counter complete the apparatus.

Lane (1936) and later Rymer (1939) have described integrators in which a gas relay valve flashes when the charge on a condenser attains a specified value, the condenser being charged by ionization current. A well-known commercial dosimeter, the "Mekapion" of Strauss is also somewhat similar, although using a vacuum valve.

The integrator used here works on the same principle; a neon tube flashes and discharges a condenser when its charge attains a certain value. Each "trip" of the mechanical counter corresponds to 4·4 r. at

the specimen. I am much indebted to Dr P. White and to Prof. J. A. Crowther for calibrating the Dosimeter in the Physics department of the University of Reading.

Doses 1, 2 and 3 received respectively 300, 600 and 900 "trips", corresponding to 1320, 2640 and 3960 r. units—less a small but undetermined loss due to absorption and scatter. The tube current was adjusted in each case so that these doses were administered in the same total time, 18 min. ± 15 sec. This is a matter of some importance, for as will be seen later, intensity appears to be one of the factors determining the amount of rejoining.

The relative values of the dosages both in the continuous and in the fractionated treatments are known with fair precision. The absolute values are subject to some uncertainty, owing to absorption and scatter in the material; but exact absolute values do not appear to be of much interest in the present work.

Experimental results

All the data obtained in the experiment are given in Table I. Each line in the table corresponds to the thirty cells from one slide, and each entry is the number of cells with the corresponding chromosome fragments. Totals for slides and for treatments are given on the right-hand side; the treatment totals are based on ninety cells each.

It will be noted that five cells with only five chromosome pieces were found; in each case it is fairly certain that the cells were not injured in smearing. It is possible that such cells cannot really exist, and that a very small fragment escaped observation. A cell with five fragments implies that a natural chromosome end has joined with a freshly broken one; the very rarity of these cells shows that such an event takes place very seldom if at all. But for the sake of consistency they are included in the analysis.

The data are plotted in Fig. 1. The questions to be answered by statistical analysis are:

(*a*) Have the three factors of varying dosage, fractionation and temperature a significant effect?

(*b*) Is the effect of dosage compatible with linearity?

(*c*) Is there significant interaction between any of the three pairs of factors, or between all three of them?

The method of Analysis of Variance (Fisher, 1936) is used to test the significance of the results. Conditions under which the error term used is valid have already been mentioned. It must be noted here that the

analysis is not strictly analogous to that of an agricultural experiment of standard layout. Although "slides" are regarded as replications, and to that extent correspond to the "blocks" of an agricultural trial, the sum of squares for slides must be included in the error term. The 24 degrees of freedom available for error include then all interaction terms which include "slides", and in addition the 2 degrees of freedom for slides themselves.

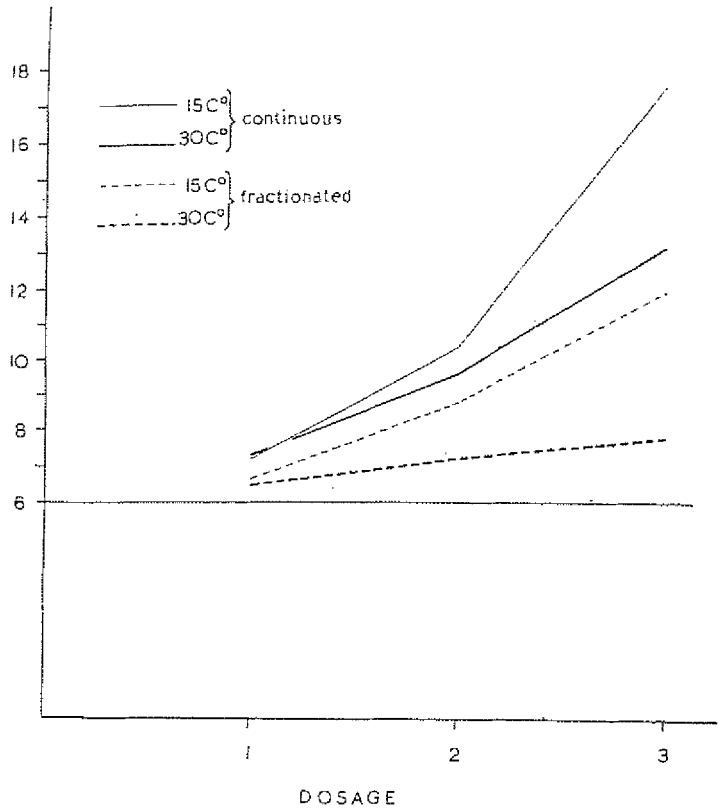

Fig. 1. Number of chromosome bodies per cell (ordinate) plotted against dosage (abscissa). Dosages 1, 2 and 3 are of 1320 r., 2640 r., and 3960 r. respectively. The actual data are given in Table I.

The main analysis is given in Table II. The variate is the sum of the chromosome pieces found in the thirty cells of each slide. Instead of the "Z" test, significance is tested by the (equivalent) variance ratio test (Fisher & Yates, 1938). It will be seen that all the three primary effects of dosage, of fractionation, and of temperature are very significant; in each case the probability of getting such effects by random sampling is less

than 1 in 1000. As the dosage variable was used at three levels, we may test whether the effect departs from linearity, by partitioning the sum of squares into a part accounted for by a linear regression, and a part not so accounted for. This remainder is not significant, and so the effect of dosage may be adequately represented as a straight line.

The dosage-fractionation interaction tests whether the effect of fractionation is different as dosage changes, i.e. that the *slope* of the continuous lines is different as a whole from the slope of the interrupted ones on the graph (Fig. 1). This is statistically very significant. In the same way as with dosage, it is possible to test if the *increase* of the frac-

TABLE II

Main analysis of variance, the sum of fragments from the thirty cells of each slide being the variate.

	Degrees of freedom	Sum of squares	Mean square	Variance ratio	Probability level: − = >0·05 + = 0·05–0·01 + + = 0·01–0·001 + + + = <0·001
Dosage:	2	176,768	88,384	58·28	+ + +
Linear regression	*1*	*172,381*	*172,382*	*113·7*	+ + +
Remainder	*1*	*4,387*	*4,387*	*2·892*	−
Fractionation	1	58,725	58,725	38·73	+ + +
Temperature	1	26,352	26,352	17·38	+ + +
D.-F.:	2	33,648	16,824	11·09	+ + +
Linear regression	*1*	*31,104*	*31,104*	*20·51*	+ + +
Remainder	*1*	*2,544*	*2,544*	*1·678*	−
D.-T.:	2	25,458	12,729	8·394	+ +
Linear regression	*1*	*23,814*	*23,814*	*15·70*	+ + +
Remainder	*1*	*1,644*	*1,644*	*1·084*	−
F.-T.	1	69	69	(21·84)	−
D.-F.-T.	2	470	235	(6·459)	−
Error	24	36,394	1,516		
Total	35	357,885			

tionation effect with dosage is linear; it will be seen that the departure from linearity is not significant. The effect of fractionating dosage, which is to reduce the number of chromosome fragments, is greatest at higher dosages.

The interaction of dosage-temperature tests if the action of higher temperature, which reduces the number of fragments, is greater at higher dosage. This is found to be so, the level of probability being between 0·01 and 0·001. Here again, the interaction is almost entirely linear, the remainder being quite non-significant.

Fractionation, on the other hand, acts independently of temperature. In other words, at any particular dosage level, the effect of any combination of fractionation and temperature may be represented as the sum of

the effects of the two factors separately. The mean square for this fractionation-temperature interaction is actually less than error, though not significantly so. It is easier to visualize the meaning of the fractionation-temperature interaction by plotting the data in a different form, with temperature as the abscissa. This has been done in Fig. 2. The non-significance of fractionation-temperature means that the slope of the continuous lines as a whole does not differ from that of the interrupted ones.

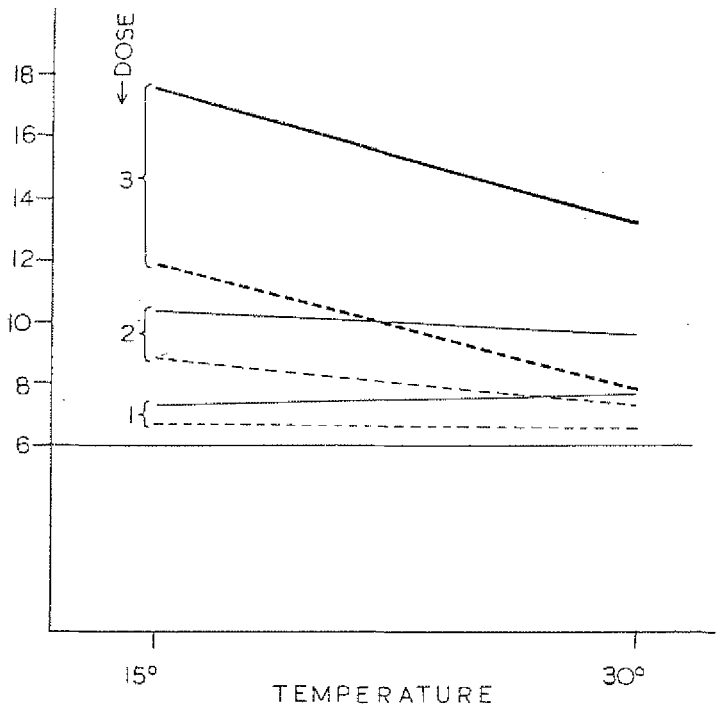

Fig. 2. The same data as in Fig. 1, but plotted with the temperature in abscissa, in order to make the meaning of the dosage-fractionation interaction clear (see text).

Finally, the triple interaction of dosage-fractionation-temperature tests the extent to which single factors and interactions between pairs of factors fail to account for the results. This mean square is also less than the error term.

The totals of each treatment, based on ninety cells, are used as the variate in the above analysis. It is of interest to find out how much of the error sum of squares is due to the variance *between cells in the same slide*. This is done in Table III. Each entry in the first column is based on 87 degrees of freedom, 29 from each slide; entries in the second column

are based on 2 degrees of freedom each. A comparison of the totals is made at the bottom of the table. It will be noted that the second total, 1212·97, is equivalent to the error term of the main analysis and has only to be multiplied by 30 for the change in variate, since in the main analysis sums of thirty cells were used.

This mean square *between* slides is very much greater than that *within* slides. Such a result is very important from the point of view of experimental technique. It shows that the variance between cells on the same slide is a grossly inadequate estimate of error, and that replication of

TABLE III

Partition of the error term

	Variate = x = number of chromosome fragments per cell		Variate = $\sqrt{(x-6)}$	
Treatment	Sum of squares within slides 87 D.F. each	Sum of squares between slides within treatments 2 D.F. each	Sum of squares within slides 87 D.F. each	Sum of squares between slides within treatments 2 D.F. each
A	843·00	91·40	18·852	1·901
B	499·27	753·23	22·486	36·053
C	443·54	47·75	36·494	3·681
D	462·71	106·28	38·075	18·787
E	177·54	18·01	43·348	4·430
F	61·54	5·08	31·183	2·254
G	580·87	101·23	22·027	4·166
H	198·47	34·69	36·270	4·038
I	453·94	10·52	37·481	0·798
J	127·27	11·35	29·485	3·235
K	198·81	30·48	44·667	7·462
L	53·54	2·95	26·873	1·678
Total	4100·50	1212·97	387·240	88·482
Degrees of freedom:	1044	24	1044	24
Mean square:	3·928	50·55	0·3709	3·687
Variance ratio:	12·87		9·939	
Probability level:	<0·001		<0·001	

slides is necessary in work of this kind. Great numbers of cells have in the past been scored without the use of replication; time spent in this work has been largely wasted.

Another problem arises in connexion with these numbers. It is evident that the components of the error term given in Table III appear heterogeneous. A test for the homogeneity of a set of variances has been given by Stevens (Fabergé, 1936). This consists, essentially, of comparing by a χ^2 test the empirical variance of the set of variances with an expected theoretical value. For the sum of squares between slides within treatments we get $\chi^2 = 506{\cdot}4$ for 11 degrees of freedom. Thus there is no doubt that great heterogeneity among the variances exists, which tends on the whole to be greater for treatments having bigger means.

Such a state of affairs makes it necessary to use caution in applying tests of significance. Had any of the effects or interactions been on the border of significance, there would have been some uncertainty about their interpretation. Fortunately, the difficulty does not arise in this particular case, since all the results fall into very clear-cut levels of probability. It is possible, nevertheless, to have another check on the validity of the error used. In some respects the triple interaction can be considered as a kind of error. Using this, with 2 degrees of freedom, for the tests of significance, results very similar to those of the main analysis given in Table II are obtained; the levels of significance attained are not always as high, but every item which is significant with the slide-replication error remains so with the triple interaction error.

TABLE IV

Analysis of variance with $\sqrt{(x-6)}$, *summed for groups of thirty cells, as variate; x being the number of fragments per cell*

	Variance ratio	Probability level: − =>0·05 + =0·05–0·01 + + =0·01–0·001 + + + =<0·001
Dosage:	69·95	+ + +
Linear regression	*139·9*	+ + +
Remainder	(*25·60*)	−
Fractionation	42·40	+ + +
Temperature	14·30	+ + +
D.-F.:	5·123	+
Linear regression	*9·887*	+ + +
Remainder	(*2·644*)	−
D.-T.:	5·036	+
Linear regression	*9·873*	+ + +
Remainder	(*5·023*)	−
F.-T.	1·244	−
D.-F.-T.	15·32	−

An attempt to diminish the heterogeneity of the error by changing the metric of the variate was made. It can be seen from Table I that the data from each slide resemble Poisson series if the number of fragments above 6 is considered. The quantities $\frac{S(x-\bar{x})^2}{\bar{x}}$ (where x is the number of fragments above 6) were calculated for each slide, and were found to be, on the whole, but slightly above $\chi^2=28{\cdot}3$, the value expected for a Poisson series. The square root transformation is suitable for data in a Poisson distribution, and the whole analysis was therefore recalculated using the square root of the number of fragments above 6, summed for each slide, as the variate. The analysis obtained was very strikingly similar to that of Table II. The tests of significance resulting from this change in metric are given in Table IV. All the conclusions remain unaltered.

A partition of the error term with $\sqrt{(x-6)}$ as the variate is given in the right-hand half of Table III. The variance between slides is still large as compared with that within slides, and the heterogeneity, although diminished, is still far too great.

The final conclusion which can be drawn from the statistical analysis is, it is thought, that the tests of significance given in Table II may safely be allowed to stand.

Discussion

The results of this experiment are perfectly clear cut as far as statistical significance is concerned. But they are by no means easy to interpret. The arrangement of the experiment cannot be blamed for this state of affairs. It was desired to know in the first instance whether the effect of X-rays on chromosomes, observed in this material by a particular technique, was capable of simple interpretation. The answer to this is in the negative.

It is clear that a simple experiment involving, for example, only dosage as the variable, would have yielded results of straightforward appearance, from which false conclusions could have been drawn. Thus, the principle of investigating several factors simultaneously is amply justified.

The different mechanisms which have been suggested for the production of chromosome aberrations by X-rays have been thoroughly discussed by Bauer *et al.* (1938) and also by Goodspeed & Uber (1939). There is therefore no need to repeat it here.

There exists no *direct* evidence that chromosome aberrations are a primary effect of radiation. But for lethals in *Drosophila* there is good evidence that the action is at least fairly direct, and that it depends on ionization and not on quanta absorption. Now if, as seems probable, an appreciable number of lethals are in fact identical with gross structural changes, we may conclude that the latter are also induced by fairly direct means. For this deduction to be true it is sufficient that an appreciable proportion of the chromosome aberrations should act as lethals; the genetic experiments are statistically quite sensitive, and would have shown if even a small part of the lethals detected obeyed rules other than those expected with direct action. For a given dose, sperms with structural changes in the *X*-chromosome are produced with a frequency which is, roughly, of the same order as the frequency of lethals. This matter has been discussed by Bauer *et al.* (1938) and by Demerec & Hoover (1936).

The evidence that lethals are produced by a direct mechanism rests on several different types of experiment, which are summarized by Timoféeff-Ressovsky *et al.* (1935) and by Stubbe (1938). One of the critical experiments is the demonstration of the absence of any effect from fractionation or dilution of the dose in time. It has been customary to deduce from this that there is no recovery in the mutation process; in fact the experiment only shows that if repair occurs, the proportion of recoveries is independent of the amount of injury. If any appreciable part of the lethals are gross structural changes, it is clear that repair does take place, for most of the viable structural changes that arise in X-rayed *Drosophila* sperm would have been lethal if the breaks had not rejoined. In *Drosophila* then, the proportion of breaks that rejoin is largely independent of the extent of the injury, and of factors such as temperature. In *Tradescantia*, on the other hand, there is a strong effect from a comparatively small fractionation of dosage, and also from a temperature difference of 15° C. There seems, therefore, to be a fundamental difference between these two organisms. This might well be due to the different state the chromosomes are in at the time of treatment. In *Tradescantia* they are dispersed in a large nucleus, and it is likely that broken ends get out of alignment before fusion can take place, all the more so if there are several breaks per thread. Chromosome bodies normally repel each other, and this will contribute to the separation of fresh ends. It will be seen later that the amount of repair is some inverse function of the dose, and the above mechanism seems adequate to account for it, several breaks per chromosome making it easier for the fresh ends to get out of alignment.

In *Drosophila* sperm, on the other hand, the chromosomes are tightly packed with little if any cytoplasm. They probably cannot move much to get out of position, and can be described as splinted. Under those conditions it is easy to imagine that the proportion of rejoins is independent of the number of breaks produced, and that fractionation of dosage is without effect. Muller and his colleagues at Edinburgh have shown that the repair of *Drosophila* chromosomes takes place not in the sperm, but at or after fertilization. (Unpublished, reported at the Seventh Genetics Congress, quoted by permission.)

The possibility should not be forgotten that X-ray action in *Tradescantia* is quite indirect. But the complications of any indirect mechanism are so great that it seems unprofitable to discuss the present data on any such supposition. In particular, the more indirect is X-ray action, the more stages there are at which recovery might act. If, on the other hand,

breakage is caused directly, then recovery must be identified with chromosome rejoining. This supposition has been made in what follows. By adopting it, the meaning of the data is considerably narrowed, and some conclusions about the rejoining process can be drawn.

It should be noted in passing that an effect of fractionation is not to be expected from the salivary gland technique, which scores points of rejoining, unless there are other complications. As far as the author is aware, no fractionation experiment has ever been analysed by the salivary gland method.

Thus it appears that the simple mechanism, in which breakage is caused by irradiation and rejoining follows independently, a view due in the first place to Stadler (1932), is one that fits the present data satisfactorily.

Of the breaks induced, some remain until observation, some rejoin to give chromosome rearrangements, and still others presumably reconstruct the old linear sequence. It is difficult to estimate the relative proportions of the two last classes, since repairs giving back the former sequence cannot be detected (except perhaps if they give rise to point mutations). As will be seen later, there are reasons for thinking that recoveries of the old structure are very numerous.

Making the simplest assumptions compatible with the data, the process of production of observable chromosome fragments may be pictured as follows.

Irradiation causes chromosome breaks in numbers proportional to dosage. Some of these breaks rejoin to reconstruct the old linear sequence, some give rearrangements, others remain as breaks and are scored.

The proportion of breaks rejoining is some *inverse function* of the number initially produced; for instance, a simple inverse proportionality can account for the results. This gives a fractionation main effect in the analysis of variance. To explain that fractionation has more effect at higher dosage (the dosage-fractionation interaction) one must suppose that this inverse function of dosage representing the amount of rejoining is in some way dependent on the total dose; the factor of intensity may also come into play (Sax, 1939).

A higher temperature produces more rejoining, in such a way (*a*) that its effect is greater at higher total doses, and (*b*) the same whether the total dose is administered continuously or in parts. The first condition (*a*) is satisfied if one supposes that a higher temperature allows greater proportion of breaks to rejoin. From the second condition (*b*), we may

deduce that the mechanism whereby a greater dose or a greater number of breaks hinders rejoining has no strong temperature coefficient.

The assumptions made above are the simplest possible, so that the situation is at best very complicated. It is possible that much more specific conclusions about the physics of chromosome repair can be deduced; the writer has not the knowledge necessary to do this. But the conditions which any theory must satisfy are set out above.

It is easier to imagine that the repair process shown by fractionation effect acts entirely within the time intervals between irradiations, and that many chromosome ends actually rejoin in the half-hour intervals; though if the rejoining is a sufficiently complex process this need not be so.

Now there is an average of 5·69 breaks per cell in the continuous low temperature, and 1·19 breaks per cell in the high temperature fractionated treatments. Even supposing that no repairs at all occurred in the former, about four breaks out of five must have rejoined in the latter treatments, probably within 3 hr., and certainly within 70 hr.

It might be supposed that free ends come into contact by chance, as a result of movement in the nucleus. But such a simple view leads to difficulties. As a rough approximation, we may consider the ends as having an "effective diameter" of, say, 2μ (which is greater than their visible size) and moving freely in a space of the same volume as a *Tradescantia* pollen nucleus. Given the number of "collisions" per unit time, we may calculate their "root mean square velocity" by the well-known method used in the Kinetic Theory of gases. Even allowing 70 hr. for four breaks out of five to rejoin, the r.m.s. velocity, $2{\cdot}7\mu$ per minute, is of the order of $5\times$ the speed of anaphase separation in this organism (Barber, 1939); with a 3 hr. time limit, it is over 1μ per second. Unless X-raying causes such a violent agitation, which normally is supposed not to occur in the resting nucleus (Belar, 1928), one must suppose either that free ends actually attract one another, giving them an effective diameter several times their apparent size, or else that a great deal of the repair consists in reconstruction of the former linear sequences. This latter view seems very plausible, and is doubtless the solution of the difficulty.

Sax (1938) could detect no effect from temperature differences at the time of irradiation. But in later work Sax & Enzmann (1939) have found an effect of an order of magnitude similar to that in our own data. Here again direct comparison is difficult owing to the different method of scoring.

Even with the scoring system used by Sax, it seems reasonably certain

that a great many "breaks" would go undetected because they have rejoined in their former position. In view of the results now presented, which demonstrate the complicated relation between the amount of repair and several other factors, we think that the evidence provided by the precise form of the dosage-effect curve *in material of this type* may be overestimated. Any attempt to determine the "sensitive volume" is still less certain. It should be noted that the form of the dosage-effect curve depends on the method of scoring; hence the necessity that the latter should be clearly definable. For example, the linear relation found for the chromosome pieces scored in this work would lead one to expect a frequency of two-break aberrations proportional to the square of the dose, if the proportion of repairs does not complicate the shape of the curve.

Summary

An experiment is described in which chromosome fragments produced by X-rays were counted in pollen grain divisions of *Tradescantia bracteata*. Twelve different treatments were used: X-ray dosage of 1320, 2640 and 3960 r., each given continuously in 18 min. or fractionated in 3 hr.; each combination of dosage and fractionation with the material at both 15 and 30° C. To get a valid estimate of error replication was used, and the data treated by Analysis of Variance. Within the fiducial limits of the experiment it is shown that:

(1) The effect of increasing dosage is linear.

(2) Fractionating the dosage reduces the number of fragments, more so at higher dosage.

(3) Higher temperature reduces the number of fragments, more so at higher dosage.

(4) The effect of fractionation is the same at both temperatures.

(5) There is no triple interaction between dosage, fractionation and temperature.

(6) By making several assumptions which at present appear reasonable, it is concluded that rearrangements are produced by the simple mechanism proposed by Stadler, i.e. that irradiation causes breaks, and rejoining takes place afterwards.

(7) If any appreciable part of *Drosophila* lethals are structural changes, then the chromosomes of *Drosophila* differ from those of *Tradescantia*. In the latter the amount of repair is influenced by the amount of injury and by temperature, in the former it is not. A mechanism which may account for this is suggested.

(8) The amount of rejoining is some inverse function (for instance an inverse proportion) of the number of breaks initially produced, and also depends on the total dose given. The mechanism by which a greater number of breaks interferes with rejoining has no strong temperature coefficient.

(9) A higher temperature allows a greater proportion of breaks to rejoin.

(10) It is thought likely that a great many "repairs" reconstruct the old sequence of chromatin, and cannot be observed cytologically.

(11) Means of overcoming various technical difficulties in connexion with dosimetry and temperature control are discussed.

(12) It is found that discrepancies between slides prepared from different buds treated alike are much greater than can be accounted for by variation within slides. The necessity for adequate replication in any quantitative work of this kind is demonstrated. Any estimate of error based on variance within slides is quite inadequate.

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