THE EFFECT OF IONIZATION DISTRIBUTION ON CHROMOSOME BREAKAGE BY X-RAYS

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(With Eight Text-figures)

Observations of Giles (1940) and Thoday (1942) have shown that neutrons are more effective per roentgen than are medium wave-length X-rays in producing all types of structural changes of chromosomes in irradiated *Tradescantia* pollen grains. This means that several ionizations are usually required to break a chromosome or a chromatid, for the distribution of the ionizations in a proton track, from neutron radiation, is many times denser than that of the ionizations in an electron track from medium X-radiation. Thus, if a single ionization were effective in each case in producing a break, we should expect either equality of effect or else a lesser efficiency on the part of the neutrons through wastage of ionizations. This wastage would result from more ionizations than necessary being produced within the confines of a particular part of the chromosome thread, the additional ionizations adding to the *dosage* without appreciably increasing the probability of breakage.

But as we have seen, medium X-rays are less efficient per ionization than neutrons, and this must come about through the number of ionizations produced in a chromosome when an electron passes through it usually being insufficient to cause a break. The density of ionization produced by an electron is not constant, more ionizations per micron of path being produced near the tail end of the path where the electron is travelling slowly than near the beginning where it is travelling quickly. It is only if this tail end of the path passes through the chromosome that sufficient ionizations are produced to cause a break. In another paper (Lea & Catcheside, 1942) we have gone into this matter, and from consideration of the relative efficiencies of neutrons and medium X-rays have been able to calculate the length of this effective tail ($0{\cdot}25\,\mu$) and the approximate number (17) of ionizations needed to produce a break.

If this theory is correct, then there should be a marked wave-length effect, i.e. a dependence upon wave-length of the number of aberrations per ionization, for X-rays of different wave-length all ionize by the intermediary of electrons, but the longer the wave-length the less energetic is the electron and the shorter its track. The effective tail is of course of the same length whatever the initial energy of the electron, providing that this is not less than the minimum required to give a complete tail. X-rays of different wave-lengths should therefore be of approximately equal efficiency *per electron*, which means that the shorter wave-lengths are less effective *per ionization* on account of the ionizations produced in the earlier, less densely ionizing, part of the track, which add to the dosage but do not contribute to the biological effect. Clearly an X-ray of wave-length such that the electron it produces has just sufficient energy to produce a complete tail will be of optimum efficiency. Still longer wave-lengths will be less efficient, since if the electron tail is too short to traverse the chromosome thread a break is unlikely.

In this way we were able, in the paper cited, to give a predicted curve for the variation of efficiency with wave-length showing a maximum efficiency in the neighbourhood of 4–5 Å. This curve we reproduce in Fig. 8 together with the experimental data we have now obtained and which it is the purpose of the present paper to describe. It is clear that the experimental points satisfactorily reproduce the essential features of the predicted curve, and thus confirm the theory on which the curve is based.

It will be noticed that the interesting part of the curve of variation of efficiency with wave-length lies in the soft X-ray region of wave-lengths exceeding 1 A. This explains why previous workers (e.g. Fabergé, 1940), using shorter waves, have failed to detect a dependence of effect upon wave-length. We have not yet made a satisfactory calculation of the course of the curve for wave-lengths shorter than about 2 A., on account of the complication introduced by δ-rays, but believe the variation with wave-length in this region to be slight, as suggested by the dotted part of the curve.

X-rays of wave-length in the range 1–10 A. with which we are concerned in the present experiment are difficult to apply to biological material owing to their low penetration, the longer waves being seriously absorbed even by 10 μ of tissue. It is possible, however, to use pollen tubes since these are thin enough to permit most of the incident energy of the soft X-rays to reach the nucleus and the loss by absorption can be estimated satisfactorily.

MATERIALS AND METHODS

Pollen of *Tradescantia bracteata*, clone 20[2], was sown on an agar-sugar-gelatin medium, following the method devised by Swanson (1940). The pollen grains were then allowed to germinate in a moist chamber in the presence of acenaphthene crystals. The effect of acenaphthene is to inhibit spindle formation and so block the pollen-tube mitosis at metaphase. The pollen germinated rapidly and the pollen tubes were irradiated between 3 and 3½ hr. after sowing, that is at a time when the generative nucleus had already passed into the pollen tube. After irradiation the slides were returned to their moist chamber with acenaphthene. They were fixed in Benda 18–20 hr. after sowing and then stained with gentian violet-iodine, according to the usual methods.

The mature pollen grain in *Tradescantia* contains two nuclei, the tube nucleus which is spherical and feebly stainable and the generative nucleus which is elongated and bent into an arc and is deeply stainable. In 3-hr.-old pollen tubes the generative nucleus has already passed into the tube. At the time of irradiation, therefore, the target presented consists of the cylindrical pollen tube of about 6 μ diameter with a centrally placed pencil-shaped nucleus of about 3 μ diameter. These measurements are of significance in estimating the actual dosage obtained within the nucleus.

RADIOLOGICAL METHODS

In these experiments X-rays of wave-lengths 0·15, 1·5, 4·1 and 8·3 A. were used. In addition, some experiments were made with α-particles. The X-rays of 0·15 A. were obtained from a therapy-type tube operating at about 160 kV. constant potential, with 0·7 mm. Cu + 1·2 mm. Al filtration. The X-rays emitted under these circumstances ranged in wave-length from about 0·08 to 0·04 A., and the figure 0·15 A. quoted is an average defined as that wave-length having the same half-value layer in copper (1·0 mm.) as the band of wave-lengths actually used.

The X-rays of wave-lengths 1·5, 4·1 and 8·3 A. were, however, practically monochromatic, being the characteristic radiations of copper (K), silver (L) and aluminium (K) respectively. These radiations are obtainable nearly pure and at high intensity from inexpensive apparatus. The X-ray tube employed was laboratory built, and this tube, and the methods of measuring the radiations from it, have been described (Lea, 1941). The exposures were made by bringing the slides up to a fixed distance (about 5 mm.) from the window of the tube. Suitable means were employed for positioning the slides accurately and rapidly, so that exposures of a few seconds' duration could be timed with reasonable precision. The area of the slide examined was confined to a region of 5–6 mm. diameter over which the radiation intensity was uniform.

The dose rate measured was that at the surface of the irradiated specimen, and in the case of the X-rays of wave-lengths 4·1 and 8·3 A., which are only feebly penetrating, it was necessary to make allowance for the diminution of intensity of the radiation in penetrating the pollen tube. The diminution was negligible in the case of the other radiations used. The pollen tubes were 6 μ in diameter, and the diminution of intensity at depths 3 and 6 μ below the surface was calculated assuming the absorption coefficients to be the same as in water, viz. 0·021 μ^{-1} and 0·14 μ^{-1} respectively. The dose rate at depth 3 μ was taken to be the effective dose rate in the experiment.

Table 1. *Physical data of radiations*

Radiation	X-rays of wave-lengths 0·15 A.	1·5 A.	4·1 A.	8·3 A.	γ-rays	
Dose rate:						
At surface	1·57	17·4	11·1	23·7	13·3	roentgen/sec.
At depth 3 μ	1·57	17·4	10·4	15·5	13·3	
At depth 6 μ	1·57	17·4	9·8	10·2	13·3	
1 roentgen =	1·70	1·30	1·54	1·52	2·63	ionizations per cubic micron in tissue of unit density
1 'energy unit' =	1·0	1·31	1·10	1·12	0·647	roentgen
Duration of exposure	30				2	sec.
	60	5	8	5	4	
	90				6	
	120				8	

The α-rays were furnished by a source of polonium deposited on a silver disk about 1 cm. in diameter, held at a distance of 2 mm. from the pollen tubes. The dose rate under these circumstances was measured by a shallow ionization chamber.

All dosages were measured in roentgen, a unit depending on ionization in air. In comparing the efficiency of different radiations it is more significant to consider ionization in the irradiated tissue. The ratio between ionization per unit volume in tissue and ionization per unit volume in air is not quite constant for different radiations, since it depends on the ratio of the absorption coefficients of the two media, which varies somewhat with wave-length. To calculate ionization in tissue from measured roentgen it is necessary, therefore, to know the absorption coefficient of the particular X-rays used in the part of the tissue in which are generated the photoelectrons which traverse the chromosomes. This means the chromosomes themselves in the case of the 8·3 A. radiation; the chromosomes and their immediate surroundings for 4·1 A.; the pollen tube as a whole for 1·5 A.; while for 0·15 A. it is partly the pollen tube, partly the agar medium below, and partly the cardboard above (a sheet of the latter being placed over the slides in the case of this radiation to enable secondary electron saturation to be achieved). In

the absence of exact knowledge of the elementary chemical analysis of the various media, the absorption coefficients were calculated in every case as if the media had the elementary composition CH_2O. For this reason, in converting roentgen into terms of energy absorption in tissue, uncertainties of 10% or even 20% are possible.

The factors for converting roentgen into ionizations per cubic micron are listed in Table 1. For shorter wave-lengths than those listed, the factor remains close to 1·70.

The most logical unit of dosage for theoretical work would probably be one ionization per cubic micron of tissue of unit density. In view, however, of the fact that the roentgen is firmly established as a unit of dose, we shall follow Gray (1939) and use as a unit of dose the 'energy unit' which represents the energy dissipation in tissue produced by 1 roentgen of hard X-rays or γ-rays, i.e. 1·7 ionizations per cubic micron according to Table 1. In reporting the results of experiments in subsequent sections, therefore, we give them not only per roentgen, but also per energy unit. The ratio between roentgen and energy units is shown in Table 1.

OBSERVATIONS

The number of divisions actually observed in irradiated pollen tubes was rather low in spite of the expenditure of a considerable amount of labour. The reason is twofold. Only pollen tubes within a circle of diameter 5 mm. could be uniformly irradiated, and the pollen grains had to be sown sparsely to secure a thin target not more than one pollen tube deep. Usually several slides for each irradiation gave a number of observations each, but it has not been thought worth while to present the data for individual slides. The slides were found to be mutually consistent, with no evidence of heterogeneity according to the χ^2 test. Thus the Ag-L slides, which gave the most extensive data, showed in respect of chromatid break production $\chi^2=4{\cdot}5$ for 8 degrees of freedom ($P=0{\cdot}8$).

Summaries of the data obtained are given in Tables 2 and 3. Table 2 records the frequencies of all the combinations of aberrations seen in nuclei that had been subjected to soft X-rays or to medium X-rays. Table 3 summarizes the gross frequencies of each kind of aberration considered independently; it also includes the few observations made on nuclei treated with α-rays.

All the aberrations observed were of the chromatid type, indicating that the irradiations were carried out when the generative nucleus had already entered upon the prophase of mitosis. Breaks of single chromatids were commonest, followed in frequency by isochromatid breaks.

Of the fifty-one isochromatid breaks produced by X-rays, forty-five showed sister reunion both in the acentric and centric fragments (Fig. 1), two showed non-reunion in both the centric and acentric fragments (Fig. 2), one showed non-reunion in its centric (Fig. 3), and three non-reunions in their acentric fragments (Fig. 4). The occurrence of 8% of failure of possible sister reunions cannot be considered different from that seen in the distal fragments (6%) at the first pollen-grain mitosis. It seems that failure of sister reunion is relatively uncommon, but that its occurrence in both the acentric and centric fragment of the chromosome is correlated. It is possible that some of the cases of proximal and distal non-reunion may be chromosome breaks possibly even of spontaneous origin, e.g. the one occurring in the 8·3 μ. radiated slides, where we expected to

Table 2. *Number observed*

Description of chromosomes in nucleus →	8·3 A. 77·7 r.	4·1 A. 83·6 r.	1·5 A. 87 r.	0·15 A. 47·2 r.	0·15 A. 94·3 r.	0·15 A. 141·5 r.	0·15 A. 188·6 r.	Control
Normal	70	42	27	12	22	34	14	433
1*c*	6	28	12	4	7	26	18	9
1c; 1c	.	15	5	.	.	.	.	.
1c + 1c	.	.	.	.	1	12	13	.
(2c)	.	3	.	.	1	1	1	.
1c; 1c; 1c	.	2	.	.	.	1	2	.
1c + 1c; 1c	.	.	.	.	.	.	1	.
1c; (2c)	.	1	1	.	.	.	2	.
1c + 1c; 1c; 1c	.	3	.	.	.	.	.	.
(2c); 1c; 1c	.	.	.	.	.	1	.	.
1c; 1c; 1c; (2c)	.	.	.	.	.	.	1	.
1*i*	1 (*NRpd*)	3 (1*NRd*)	2	1	2	5	4	2
1*i*; 1c	.	6	2	1 (*NRpd*)	1	2	2	.
1*i* + 1c	.	.	.	.	.	2	.	1
(1*i* + 1c)	.	1	.	.	.	.	.	.
1*i*; 1c; 1c	.	1	.	.	1	2	1 (*NRpd*)	.
1*i*; 1c + 1c	.	.	.	.	.	.	1	.
1*i* + 1c; 1c	.	1 (*NRp*)	.	.	.	1	.	.
(1*i* + 1c); 1c	.	.	.	.	.	.	2 (1*NRd*)	.
(1*i* + 1c) + 1c	.	1	.	.	.	.	.	.
1*i* + 1c; 1*i*; 1c; 1c	.	1	.	.	.	.	.	.
1*i* + 1c; 1c; (2c)	.	.	.	.	.	.	1	.
1*et*	.	2	1 (*NR*)	.	.	2	2	.
1*dt*	.	2	.	.	.	1	1	.
1*et*; 1c	.	.	.	.	.	1	.	.
1*et* + 1c	.	.	.	.	.	.	1	.
1*et*; 1c; 1c	.	.	.	.	.	.	1	.
1*dt*; 1c; 1c	.	.	.	.	.	1	.	.
1*dt*; (2c)	.	1	.	.	.	.	.	.
1*et*; 1c; 1c; 1c	.	1	.	.	.	.	.	.
1*dt* + 1*dt* (3c); 1c	.	.	.	.	.	.	1	.
1*et*; 1*i*; 1c	.	.	.	.	.	1	.	.
1*dt* + 1c; 1*i*	.	.	.	.	.	.	1	.
1*euc.inv.*	.	.	.	.	.	.	1	.
1*euc.inv.*; 1c	.	.	.	.	.	.	1 (*NR*)	.
1*euc.ring*; 1c	.	.	.	.	1	.	.	.
1*euc.ring*; 1c; 1c; 1c	.	.	.	.	.	1	.	.
1*dys.dupl.dfy.*	.	.	.	.	.	1	.	.
1*dys.inv.*; 1c	.	.	.	.	.	.	1	.
Total nuclei	77	114	50	18	36	95	73	445

Key

c = chromatid break.
i = isochromatid break.
et = eucentric interchromosal interchange.
dt = dyscentric interchromosal interchange.
euc.inv. (or *ring*) = interarm intra-chromosal interchange.
dys.inv. (or *dupl.dfy.*) = intra-arm intra-chromosomal interchange.
NR = non-reunion.

In isochromatid breaks: *NRp* = proximal *NR*; *NRd* = distal *NR*; *NRpd* = proximal and distal *NR*.

Two symbols separated by a semicolon (;) means the aberrations occur in separate chromosomes, or groups of chromosomes where two or more are held together by an interchange.

Two symbols separated by a plus sign (+) means the aberrations are in separate arms of one chromosome or of a group of chromosomes held together by an interchange. Thus Fig. 3 is 1*i* + 1c and is also *NRp*.

Symbols enclosed within brackets ( ) refer to aberrations affecting the same arm of a chromosome.

find none at all.* Alternatively, we may suppose either that some parts of the chromatids do not rejoin so easily as other parts or else that some parts of the ion track, especially the denser parts, cause so much damage in breaking the chromosomes that reunion is rendered less likely. In support of the latter supposition we have the fact that three out of the thirteen isochromatid breaks due to α-rays showed non-reunion, while six only out of the fifty-one due to X-rays showed non-reunion. The α-rays are extremely densely ionizing and may be expected to cause serious damage where their tracks traverse the chromosomes.

Table 3

Nature of radiation	Dose: Roentgen	Dose: 'Energy units'	Total nuclei	Chromatid breaks	Isochromatid breaks*	Interchanges: Intra-chromosomal: Inter-arm	Interchanges: Intra-chromosomal: Intra-arm	Interchanges: Inter-chromosomal: Eu-centric	Interchanges: Inter-chromosomal: Dys-centric
X-rays 8·3 A.	77·7	69·4	77	6	1 (1*NRpd*)	.	.	.	.
X-rays 4·1 A.	83·6	76·0	114	106	15 (1*NRp*) 4(1*NRd*)	.	.	3	3
X-rays 1·5 A.	87·0	66·4	50	27	4	.	.	1	.
X-rays 0·15 A.	47·2	47·2	18	5	2 (1*NRpd*)	.	.	.	.
	94·3	94·3	36	15	4	1	.	.	.
	141·5	141·5	95	76	13	1	1	4	2
	188·6	188·6	73	87	12 (1*NRd*) (1*NRpd*)	2	1	4	4
α-rays	26·6	41·1	11	3	3	.	.	.	.
	53·2	83·2	1	0	0	.	.	.	.
	79·8	123·3	22	28	7 (2*NRpd*)	.	.	1	2
	106·4	164·4	11	27	3 (1*NRpd*)	.	.	.	.

* *NRp* = non-reunion proximal. *NRd* = non-reunion distal. *NRpd* = non-reunion proximal and distal.

Interchanges, both intra- (six cases) and interchromosomal (twenty-one cases), were observed except in the Al *K*-radiated slides. The relative proportion of these two classes is about the same as we have found (namely 112 intra- : 388 interchromosomal) at the first pollen-grain division. There is an indication of a relative excess of intra-chromosomal interchanges over expectation (one-fifth of interchromosomal interchanges), in agreement with that found by Newcombe (1942) in various organisms.

There were twelve eucentric to nine dyscentric interchanges, a proportion not significantly deviating from the expected equality. Again there were four intra-chromosomal interchanges with breaks in opposite arms to two with both breaks in the same arm.

* We have obtained a further series of data on chromatid and isochromatid production by Al *K*-radiation (wave-length 8·3 A.). No isochromatid breaks were found, only chromatid breaks being seen. The numerical results are summarized in the following table:

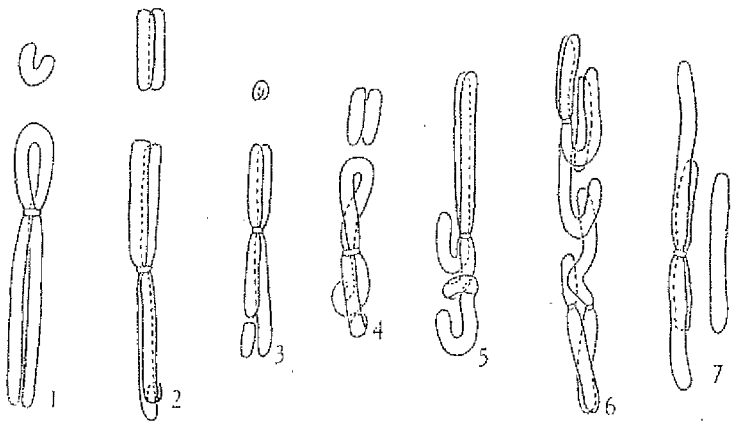
Dose: r.	Dose: e.	Total nuclei	Chromatid breaks	Yield per cell: per r.	Yield per cell: per e.
169	151	83	8	$0{\cdot}57 \pm 0{\cdot}2 \times 10^{-3}$	$0{\cdot}64 \pm 0{\cdot}2 \times 10^{-3}$
432	386	21	5	$0{\cdot}55 \pm 0{\cdot}2 \times 10^{-3}$	$0{\cdot}62 \pm 0{\cdot}2 \times 10^{-3}$

The yield of chromatid breaks in the new series is lower than in the old, but not significantly different from it. The joint yield for all the different doses is $0{\cdot}654 \pm 0{\cdot}15 \times 10^{-3}$ per cell per roentgen and $0{\cdot}731 \pm 0{\cdot}17 \times 10^{-3}$ per cell per energy unit.

The absence of isochromatid aberrations in the new series, in which four such aberrations would have been expected on the basis of the first experiment, strengthens the belief that the single one observed in the first experiment was a spontaneous occurrence. Retaining it and grouping all the data for the three doses, we have a yield of isochromatid breaks of $3{\cdot}4 \pm 3{\cdot}4 \times 10^{-5}$ per roentgen per cell and $3{\cdot}8 \pm 3{\cdot}8 \times 10^{-5}$ per energy unit per cell.

These new observations are incorporated in Fig. 8.

One of the latter was a duplication-deficiency configuration (Fig. 5), a type which we have not been able to recognize for certain in *Tradescantia* pollen-grain divisions. The greater ease of recognition in the pollen-tube division is no doubt due to the lesser contraction of the chromosomes as compared with the pollen-grain division.



Figs. 1–7. Chromatid aberrations induced by X-rays in the pollen-tube division of *Tradescantia*. Figs. 1–4, isochromatid aberrations; Fig. 5, intra-chromosome duplication deficiency; Fig. 6, incomplete eucentric interchange; Fig. 7, incomplete eucentric ring. For descriptions, see text. ×2000.

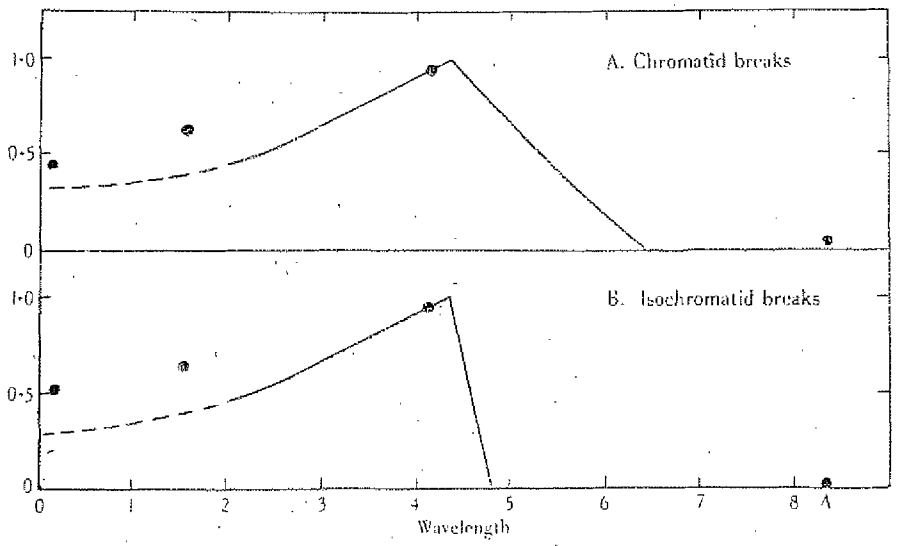


Fig. 8. Yields of chromatid and isochromatid breaks, as functions of X-ray wave-length, for equal ionization in the tissue. Curves theoretical, points experimental, scaled to agree at 4·1 Å.

No doubt the present records are highly heterogeneous in source, but it is of some interest that the proportion of incomplete interchanges (7·4%) is about the same as at the first pollen-grain mitosis 24 hr. after X-raying (6·0%, unpublished) and after neutron radiation (6·5%—Thoday, 1942). Two cases of incomplete interchanges, each with one failure of the two possible non-sister reunions, were seen. In one case, the reunion failure was between acentric and centric fragment chromatids forming part of a eucentric interchange (Fig. 6). In the other, the broken ends of the centric fragment of what would have been the ring chromatid of a eucentric ring configuration had failed to rejoin (Fig. 7).

Generally speaking, then, there is no noticeable qualitative difference between the chromatid aberrations seen in the pollen-tube and pollen-grain divisions. Nor is there any detectable difference between the two divisions *within* the classes of aberrations (chromatid, isochromatid and interchange) in respect to the proportions of the various types belonging to them. This is satisfactory, since we shall see that the proportions of the three classes are different at the two divisions and that the differences may be ascribed to the different geometry of the chromosomes, arranged in a cylindrical rather than a spherical nucleus, at the time of irradiation.

Analysis of the results

There is adequate reason, both experimental and theoretical, for believing that the yields of chromatid and isochromatid breaks per cell produced by X-rays are proportional to the first power of dose, and that the yield of interchanges is, when corrected to infinite intensity, proportional to square of dose, while with α-ray the yields of all three types of aberration are proportional to first power of dose. We have not therefore in these experiments usually obtained data at several different dosages, but have instead reduced the labour of the experiment by employing a single dose only, and determining the coefficient k or m as the case may be in the formula $y=mx$ or $y=kx^2$, where y is the yield of aberrations per cell, and x is the dose. In those cases, namely, medium X-rays and α-rays, where several doses were used, we were able by means of the χ^2 test to check that the equations assumed satisfactorily fitted the data, but on account of the small numbers of aberrations the test was not a critical one.

Since with soft X-rays the longest exposure was 8 sec., the yields of interchanges may be fitted directly to the $y=kx^2$ formula. With medium X-rays the exposures extended up to 2 min. and the equation $y=kx^2G$ of the time-intensity theory (Lea & Catcheside, 1942) was used with $\tau=3{\cdot}3$ min. as in microspores. The correction so made is small, and no serious error will arise in the event of τ being different in pollen tubes, unless it is considerably smaller than 3·3 min.

The yields of the various types of aberration are tabulated in Table 4. The errors shown are based simply on the square root of the number of aberrations scored, and are to be interpreted in accordance with the considerations advanced by Stevens (1942). A check was made to verify that no additional error occurred due to variations between slides in excess of that due to the finite number of aberrations scored.

For comparison also we show figures for medium X-rays and α-rays on microspore chromosomes, the medium X-ray data being that of Thoday (1942, cp. Lea & Catcheside, 1942, Tables I and III), while for the α-ray data we are indebted to Gray & Kotval (unpublished).

In Fig. 8 A, B, the observed results for the various X-ray wave-lengths are plotted on the theoretical curve, the observed results having been scaled so that the 4·1 A. results are exactly on the curve. It should be noted that the coefficients used for plotting are those 'per-energy-unit', since it is believed that the energy unit gives a more exact measure of the actual ionization in the nucleus than does the roentgen. It will readily be seen that the results agree remarkably well with expectation, except that some aberrations were produced by the softest radiation (8·3 A.) which was expected to produce none.* But we should recall that the estimation of the effects produced is based on two

* See also footnote to p. 191.

approximations: (1) that less than seventeen ionizations within a chromosome or chromatid thread fail to break it, while seventeen or more invariably do break it, and (2) that these ionizations should be spread right across the thread. The truth is, of course, that the probability of breakage by less than seventeen ionizations, or by a train of ionizations not completely traversing the chromosome, is not actually zero. Unfortunately, we have no means at present of estimating the probabilities of breakage by

Table 4. *Coefficients of production of various aberrations induced in prophase chromosomes in the pollen-tube and pollen-grain mitosis*

	Radiation	Wave-length	Coefficient of aberration production per cell: Per roentgen	Per energy unit
	Chromatid breaks fitted to $y=mx$			
Pollen-tube division:	Medium X-ray	0·15	$5{\cdot}82 \pm 0{\cdot}43 \times 10^{-3}$	$5{\cdot}82 \pm 0{\cdot}43 \times 10^{-3}$
	Cu-*K*	1·5	$6{\cdot}20 \pm 1{\cdot}2 \times 10^{-3}$	$8{\cdot}1 \pm 1{\cdot}6 \times 10^{-3}$
	Ag-*L*	4·1	$11{\cdot}0 \pm 1{\cdot}1 \times 10^{-3}$	$12{\cdot}1 \pm 1{\cdot}2 \times 10^{-3}$
	Al-*K*	8·3	$1{\cdot}0 \pm 0{\cdot}4 \times 10^{-3}$	$1{\cdot}1 \pm 0{\cdot}5 \times 10^{-3}$
	α-rays	—	$17{\cdot}7 \pm 2{\cdot}3 \times 10^{-3}$	$11{\cdot}4 \pm 1{\cdot}5 \times 10^{-3}$
Pollen-grain division:	Medium X-ray	0·15	—	$7{\cdot}26 \pm 0{\cdot}78 \times 10^{-3}$
	α-rays	—	—	$10{\cdot}5 \pm \times 10^{-3}$
	Isochromatid breaks fitted to $y=mx$			
Pollen-tube division:	Medium X-ray	0·15	$9{\cdot}86 \pm 1{\cdot}8 \times 10^{-4}$	$9{\cdot}86 \pm 1{\cdot}8 \times 10^{-4}$
	Cu-*K*	1·5	$9{\cdot}2 \pm 4{\cdot}6 \times 10^{-4}$	$12{\cdot}0 \pm 6{\cdot}0 \times 10^{-4}$
	Ag-*L*	4·1	$15{\cdot}8 \pm 4{\cdot}0 \times 10^{-4}$	$17{\cdot}4 \pm 4{\cdot}4 \times 10^{-4}$
	Al-*K*	8·3	$1{\cdot}7 \pm 1{\cdot}7 \times 10^{-4}$	$1{\cdot}9 \pm 1{\cdot}9 \times 10^{-4}$
	α-rays	—	$39{\cdot}8 \pm 11{\cdot}0 \times 10^{-4}$	$25{\cdot}8 \pm 7{\cdot}1 \times 10^{-4}$
Pollen-grain division:	Medium X-ray	0·15	—	$27{\cdot}1 \pm 2{\cdot}0 \times 10^{-4}$
	α-rays	—	—	$115{\cdot}0 \pm \times 10^{-4}$

Interchanges fitted to $y=mx$ (α-rays) or $y=kx^2$ (X-rays)

	Radiation	Coefficient of interchange production per cell: m per roentgen	k per roentgen^2	m per energy unit	k per energy unit^2
Pollen-tube division:	Medium X-ray	—	$4{\cdot}84 \pm 1{\cdot}1 \times 10^{-6}$	—	$4{\cdot}84 \pm 1{\cdot}1 \times 10^{-6}$
	Cu-*K*	—	$2{\cdot}64 \pm 2{\cdot}6 \times 10^{-6}$	—	$4{\cdot}5 \pm 4{\cdot}5 \times 10^{-6}$
	Ag-*L*	—	$7{\cdot}54 \pm 3{\cdot}0 \times 10^{-6}$	—	$9{\cdot}19 \pm 3{\cdot}7 \times 10^{-6}$
	Al-*K*	—	—	—	—
	α-rays	$9{\cdot}17 \pm 5{\cdot}4 \times 10^{-4}$	—	$5{\cdot}93 \pm 3{\cdot}5 \times 10^{-4}$	—
Pollen-grain division:	Medium X-ray	—	—	—	$18{\cdot}1 \pm 2{\cdot}1 \times 10^{-6}$
	α-ray	—	—	$32{\cdot}0 \pm \times 10^{-4}$	—

1, 2, 3, ..., n ionizations within a chromosome, and we are, therefore, reduced to the simplifications upon which the theoretical curve connecting wave-length with the efficiency of breakage per ionization was based. The relatively close agreement between observation and expectation provides strong support for our theory.

The α-ray results we do not discuss in detail in this paper. These radiations are even more densely ionizing than the protons produced by neutrons. Since neutrons are more effective per ionization than medium X-rays (Thoday, 1942), at first glance one would be tempted to imagine that α-rays would be still more efficient. However, we have reason to believe that a proton traversing a chromosome thread almost certainly causes a break (Lea & Catcheside, 1942). The extra ionization produced by the α-rays, contributing to the dosage but not materially increasing the probability of breakage, should by the usual argument render α-rays less efficient per ionization than neutrons. The yield with α-rays should, therefore, be comparable with that given by medium X-rays. Actually α-rays appear to be more effective than X-rays. The discussion of this discrepancy is best deferred until the full data of Gray & Kotval are available.

We may compare (Table 5) the aberration coefficients of pollen-tube and pollen-grain divisions for medium X-rays and α-rays.

Table 5

Aberration	Medium X-rays	α-rays	Weighted mean
Chromatid breaks	$0{\cdot}803 \pm 0{\cdot}10$	$1{\cdot}08 \pm 0{\cdot}15$	$0{\cdot}90 \pm 0{\cdot}1$
Isochromatid breaks	$0{\cdot}364 \pm 0{\cdot}072$	$0{\cdot}224 \pm 0{\cdot}055$	$0{\cdot}27 \pm 0{\cdot}05$
Interchanges	$0{\cdot}267 \pm 0{\cdot}08$	$0{\cdot}185 \pm 0{\cdot}11$	$0{\cdot}24 \pm 0{\cdot}07$

It will be noticed that the ratio pollen tube : pollen grain for each type of aberration is approximately the same for the two kinds of radiations. Hence the difference between ease of production of the different aberrations is a characteristic of the aberration and not of the radiation used.

Reference to Table 4 will show that the coefficients of chromatid break production per roentgen for a given radiation are approximately equal for the pollen-grain and pollen-tube divisions. Thus, for medium X-rays the coefficients, multiplied by 10^3, are respectively $7{\cdot}25 \pm 0{\cdot}78$ and $5{\cdot}82 \pm 0{\cdot}43$. This means that the value f (Lea & Catcheside, 1942), which is the ratio of the number of chromatid breaks observed to the number formed, is the same in each case. The conditions surrounding chromatid break restitution are not different in the two kinds of nuclei, in spite of their different shapes and external physiological conditions.

There are relatively fewer isochromatid aberrations, however, in the pollen-tube division. Roughly they are only about one-quarter as frequent as in the pollen-grain division. This could be accounted for by supposing that the chromatids are a little farther apart at the time of irradiation, thus diminishing both the probability that an ionizing particle which passes through a specified chromatid shall pass through its sister and the probability of sister reunion of the broken ends. There seems no possibility of observing an actual difference in separation of the chromatids in the two kinds of nuclei, since the order of magnitude of the distance separating them at the critical stage is similar to or below that of the lower limit of optical resolution. We have seen earlier that there is no evidence of a clear-cut difference in the proportion of non-reunion of broken ends of isochromatid breaks in the pollen-tube and pollen-grain divisions. The probability of sister reunion of the broken ends is, therefore, not appreciably reduced in the pollen-tube nuclei and we may ascribe all the reduction in isochromatid breakage frequency to the geometrical factor of separation reducing the chance of the effective part of one ionization path traversing both the sister chromatids.

It is also clear that there is a relative reduction, to about a quarter, in the frequency of interchanges in the pollen-tube divisions. Ignoring the different shape of the nuclei in the pollen grain and the pollen tube, this reduction would suggest that the chromosomes are farther apart. The greater separation of the original breaks that would be entailed should mean a reduction in the chance of reunion of non-sister breakage ends. According to the interchange theory we have developed (Lea & Catcheside, 1942) the coefficient of interchange production should be proportional inversely to the volume of the nucleus, if the factor h is the same. Now h is the critical distance within which two breaks must be produced in order to interchange. To have four times the volume of a pollen-grain nucleus of 12μ diameter, a pollen-tube nucleus of 3μ diameter would need to be about 512μ long. Manifestly we are on the wrong track since the pollen-tube

nucleus is little more than 80 μ long at the stage irradiated, and it has a volume smaller if anything than that of the pollen-grain nucleus.

It appears then that the factor at work is not so much the spacing of the chromosomes in a greater volume as their disposition within a space of different shape. The pollen-grain nucleus is a sphere, the pollen-tube nucleus a cylinder. Moreover, the chromosomes are arranged more or less tandem in the pollen-tube nucleus, so that the proportion of the length of a chromosome thread that lies near to another part of the chromosome thread or to a different chromosome is reduced compared with that in the pollen-grain nucleus. The reduction factor merely means that only about a quarter as many breaks, per roentgen, are produced within the critical distance h of one another in the pollen-tube nucleus as in the pollen-grain nucleus. The reduction is a geometrical property of the different packing and arrangement of the chromosomes and does not imply any fundamental difference in the other conditions governing the probability of non-sister reunion.

SUMMARY

1. The coefficients of chromatid breakage (compare Table 4) are highest with Ag L-radiation ($\lambda = 4{\cdot}1$ A.) and fall off through Cu K-radiation ($\lambda = 1{\cdot}5$ A.) to medium X-rays ($\lambda = 0{\cdot}15$ A.) and to Al K-radiation ($\lambda = 8{\cdot}3$ A.) where they are least.

2. This is interpreted to mean that only the densely ionizing 'tails' of the electron tracks are effective in chromosome breakage, and that the 'tails' have a higher efficiency only where they traverse the chromatid; for the track of an Al K-electron which is shorter than a chromatid diameter is relatively inefficient.

3. The probability of survival of chromatid breaks is the same in the pollen-tube nucleus as in the pollen-grain nucleus.

4. The two chromatids of a chromosome are in contact 24 hr. before metaphase in the pollen-grain nucleus. They are slightly separated 15 hr. before metaphase in the pollen-tube nucleus.

5. The probability of interchange in the pollen-tube nucleus is reduced by the different method of packing of the chromosomes in a long cylindrical nucleus as compared with the spherical one of the pollen grain.

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