THE PRODUCTION OF CHROMOSOME STRUCTURAL CHANGES IN *TRADESCANTIA* MICROSPORES IN RELATION TO DOSAGE, INTENSITY AND TEMPERATURE

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(With Five Text-figures)

1. Introduction

In the preceding paper (Catcheside, Lea & Thoday, 1945, referred to subsequently as Paper I), exchanges and isochromatid breaks were each analysed into several subtypes. It appeared that the relative proportions of the different subtypes of exchange or of isochromatid break did not depend appreciably on the dose of radiation or on its intensity or, usually, on the temperature. In the present paper, therefore, in which we discuss the manner in which the yields of structural changes induced by X-rays depend upon dose, intensity, and temperature, we shall not need to make the numerous subdivisions employed in Paper I, but shall simply deal with the total numbers of chromatid breaks, isochromatid breaks, *c/c* intrachanges, and *c/c* interchanges. These totals will be found in Table 1 of Paper I.

Chromatid breaks are believed to be produced by the passage of an ionizing particle through the chromatid thread. Being single event processes, their yield is expected to be proportional to dose and, for a given dose, independent of intensity. Since the primary effects of radiation are independent of the temperature, the number of chromatid breaks primarily produced by a given dose is expected to be independent of the temperature. It appears that the proportion which fail to restitute does not change greatly with change of temperature (Paper I, Tables 14 and 15), so that the number of chromatid breaks observed will also not be greatly dependent upon temperature.

Chromatid exchanges involve the production of two breaks, each of the breaks requiring the passage of an ionizing particle through the chromatid concerned. It may happen that both chromatid breaks are produced by the same ionizing particle. The yield of these '1-hit' exchanges will increase linearly with the dose, and the yield for a given dose will be independent of intensity. On the other hand, it may happen that the two chromatid breaks taking part in an exchange are produced by separate ionizing particles. In this event they will not be produced simultaneously. Two breaks which are favourably located in space to exchange may fail to exchange if produced several minutes apart in time, owing to the first having restituted before the second is produced (Sax, 1939). Thus the yield of '2-hit' exchanges produced by a given dose will be diminished if the duration of the exposure is increased (with corresponding reduction of the intensity).

A given break will only take part in exchange if a second break is produced in its proximity. The probability that a second break will be produced by an independent ionizing particle within a stated distance of the first break is proportional to the dose,

hence the *proportion* of breaks which take part in 2-hit exchanges is proportional to the dose, and so the yield of 2-hit exchanges is proportional to the square of the dose. This derivation implicitly involves the assumption that the probability of any given break exchanging is much less than unity, which is valid for the range of doses commonly used. Also, its neglect of the time factor means that for the square law to hold the dose must either be given in a short time (making the probability small of restitution occurring between the production of the two breaks) or in a time which is the same for all doses (making the loss by restitution independent of the dose).

Thus the total yield of exchanges is in general the sum of two components, a 1-hit group the yield of which is proportional to the dose and, at a given dose, independent of intensity, and a 2-hit group the yield of which is proportional to the square of the dose (for different doses given in constant time) and at a given dose diminishes with increase of the duration of the exposure. Using the range of doses commonly employed, exchanges are predominantly 1-hit in neutron and α-ray experiments, while 2-hit exchanges predominate in X-ray and γ-ray experiments. (The reasons for this have been discussed by Lea & Catcheside, 1942.) The influence of the 1-hit component, however, becomes appreciable in X-ray experiments conducted at low intensity, and one of the objects of the present series of experiments was to analyse the total yield of exchanges into 1-hit and 2-hit components.

Isochromatid breaks are asymmetrical exchanges between breaks at about the same locus in sister chromatids. Since the sister chromatids are separated by a distance of the order of $0{\cdot}1\,\mu$, while breaks which typically take part in exchange are separated at the moment of formation by a distance of the order of $1\,\mu$ (Lea & Catcheside, 1942; also the present paper, § 5), the two breaks constituting an isochromatid break are, even with X-rays, more likely to be produced by the same ionizing particle than by separate ionizing particles. However, a proportion of isochromatid breaks should in principle be constituted by two breaks produced by separate ionizing particles, and this proportion, if appreciable, should be revealed by the yield of isochromatid breaks increasing slightly more rapidly than the first power of the dose and, at a given dose, diminishing slightly with increase of exposure time.

2. Chromatid breaks

We supplement the data given in Table 1 of Paper I by making use of Table 1 of Thoday (1942), and show in Fig. 1 the yield of chromatid breaks at 20° as a function of the dose. These aberrations are more difficult to score than isochromatid breaks and exchanges, and the irregularities of the points are attributed to observational errors.

Table 1 below shows that there is no variation outside the possible experimental error of the yield of chromatid breaks produced by 150 r. at a given temperature when the exposure time is varied 16-fold. Table 2 shows that the yield is, within the error, the same at 1° and 20°. It is a little lower at 30°, but the fall at 30° is much less marked than with the other types of aberrations.

3. Exchanges

The 1943 experiments (see Paper I, Table 1) include one set of four doses given in a constant time of 251 min., and a second set of four doses given in a constant time of 1·2 min. These are to be analysed into 1-hit and 2-hit components by fitting the yield

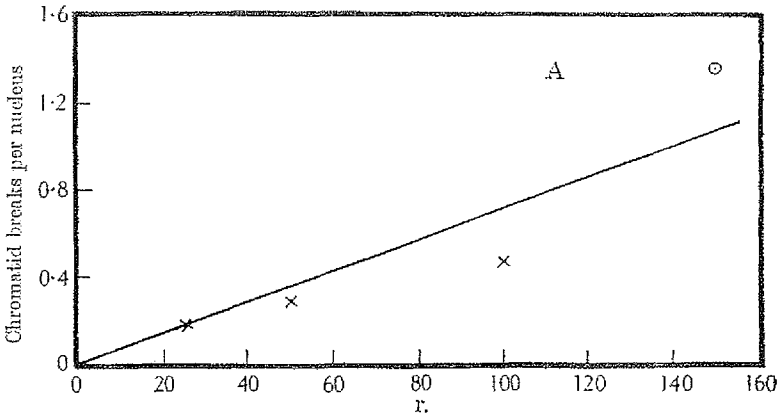

Fig. 1. Yield of chromatid breaks as a function of the dose at room temperature. × Thoday (1942); ⊙ present experiments (1940 series).

Table 1. *Aberrations per nucleus produced by* 150 *r. of X-rays*

Temp. °C.	Series	Exposure time min.	Normal cells	Chromatid breaks	Isochromatid breaks	c/c intrachanges	c/c interchanges
1	1940	1·97	0·052±0·01	1·213±0·07	0·528±0·05	0·152±0·02	0·493±0·04
		3·87	0·053±0·01	1·358±0·08	0·428±0·04	0·166±0·03	0·456±0·04
		7·77	0·063±0·01	1·322±0·06	0·510±0·04	0·116±0·02	0·399±0·03
		15·5	0·066±0·01	1·297±0·06	0·454±0·04	0·108±0·02	0·405±0·04
		31·4	0·077±0·02	1·290±0·07	0·433±0·04	0·093±0·02	0·336±0·04
20	1940	0·983	0·082±0·02	1·390±0·06	0·393±0·04	0·089±0·02	0·355±0·03
	1943	1·2	—	—	—	—	0·317±0·02
	1940	1·97	0·095±0·03	1·448±0·11	0·353±0·05	0·079±0·02	0·277±0·04
	1943	3·83	—	—	—	—	0·204±0·01
	1940	3·87	0·108±0·02	1·357±0·09	0·322±0·04	0·068±0·02	0·286±0·04
	1940	7·77	0·141±0·02	1·260±0·07	0·327±0·04	0·066±0·02	0·211±0·03
	1940	15·5	0·105±0·02	1·361±0·08	0·343±0·04	0·080±0·02	0·253±0·03
	1943	251	—	—	—	—	0·125±0·01
30	1940	0·485	0·260±0·03	0·942±0·07	0·123±0·03	0·028±0·01	0·130±0·03
		0·983	0·296±0·03	0·960±0·07	0·152±0·03	0·015±0·01	0·106±0·02
		1·97	0·273±0·03	1·031±0·08	0·123±0·03	0·018±0·01	0·121±0·03
		3·87	0·268±0·03	1·061±0·08	0·143±0·03	0·012±0·01	0·099±0·02
		7·77	0·223±0·03	1·159±0·09	0·171±0·04	0·042±0·02	0·141±0·03

Table 2. *Yields of chromatid and isochromatid breaks and c/c interchanges produced by* 150 *r. of X-rays*

Temp. °C.	Chromatid breaks per nucleus	Isochromatid breaks per nucleus	c/c interchanges per nucleus at high intensity
1	1·298±0·03	0·475±0·02	0·52
20	1·356±0·03	0·353±0·02	0·37
30	1·024±0·03	0·141±0·01	0·12

of c/c interchanges per cell (y) produced by dose x to the formula $y=\alpha x+\beta x^2$. The coefficients α and β are obtained from the experimental values of x and y by the least squares method, using the formulae:

$$\alpha=(\Sigma px^4\Sigma pxy-\Sigma px^3\Sigma px^2y)/D\pm\sqrt{(\Sigma px^4/D)},$$

$$\beta=(\Sigma px^2\Sigma px^2y-\Sigma px^3\Sigma pxy)/D\pm\sqrt{(\Sigma px^2/D)},$$

where

$$D=\Sigma px^2\Sigma px^4-(\Sigma px^3)^2.$$

In these formulae p is the weight to be ascribed to the experimental observation x, y. The observation y interchanges per cell is based on the counting of ny interchanges in n cells. In view of the fact that the frequencies with which cells containing 0, 1, 2, etc., interchanges are found after irradiation by a given dose have been found experimentally to be satisfactorily fitted by the Poisson distribution (Paper I, Table 9), and in view of the satisfactory result of the homogeneity tests between slides in the 1943 experiments (Paper I, Table 13), we may ascribe a standard deviation $\sqrt{(ny)}$ to the observation ny. The standard deviation of y is thus $\sqrt{(y/n)}$, which may be written $1/\sqrt{p}$, where $p=n/y$ is the weight of the observation x, y. It is this value of p which is to be inserted in the equations for α and β. The values of α and β obtained in the two experiments with X-rays are set out in Table 3.

Table 3. *Analysis of the X-ray dose-curves at* 20° *into* 1-*hit and* 2-*hit components*

($y=\alpha x+\beta x^2$ is the number of c/c interchanges per nucleus produced by a dose of x roentgens.)

Duration of exposure min.	α $\times 10^{-4}$ per r.	β $\times 10^{-6}$ per r.^{2}	Result of χ^2 test		
			χ^2	D.F.	P
251	2·66±0·91	3·89±0·92	2·18	2	0·36
1·2	1·85±1·04	13·06±1·19	1·98	2	0·37

The χ^2 test, the results of which are summarized in Table 3, shows that the equation $y=\alpha x+\beta x^2$ satisfactorily fits the observations. Fig. 2 illustrates the analysis by showing graphically the 1-hit and 2-hit dose curves and their sum. It is seen that the 1-hit interchanges make a serious contribution to the total yield of interchanges in the 251 min. experiment (Fig. 2A). In experiments extending over a much shorter time,

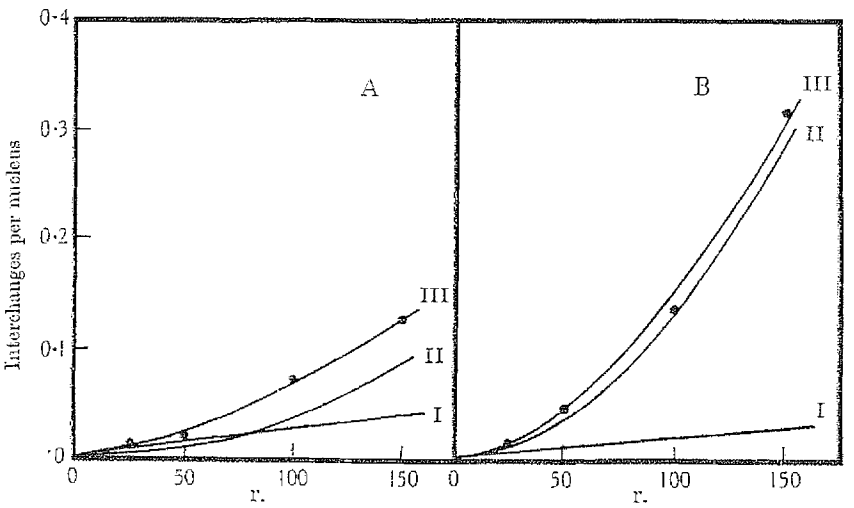

Fig. 2. Analysis of interchange dose-curve into 1-hit and 2-hit components. A, constant exposure time 251 min.; B, constant exposure time 1·2 min. Curves: I, 1-hit component; II, 2-hit component; III, sum of I and II.

as is the more usual practice, 1-hit interchanges make only a small contribution (Fig. 2B). Their effect has, however, been noticed in the past; Sax (1940) found that his chromatid exchange data best fitted the curve (dose)$^{1\cdot9}$, though quite compatible with (dose)^2.

It is evident from Table 3 that there is no significant difference between the values

of α obtained in the long-duration and in the short-duration experiments. The difference between the values of β, however, is highly significant. It follows that, as anticipated in the introduction, the reduction in the yield of interchanges obtained by increase of the duration of the exposure time is entirely due to a reduction in the yield of 2-hit interchanges, 1-hit interchanges not being affected. We shall take $(2{\cdot}3 \pm 0{\cdot}7) \times 10^{-4}$ interchanges per cell per roentgen to be the yield of 1-hit c/c interchanges at 20°, this being the mean of the two estimates of α. By subtracting $150 \times 2{\cdot}3 \times 10^{-4} = 0{\cdot}035 \pm 0{\cdot}01$ from the figures of c/c interchange yields at 20° given in Table 1 we are able to deduce the yield of 2-hit c/c interchanges produced by a dose of 150 r. These yields are plotted against the duration of the exposure time in Fig. 4.

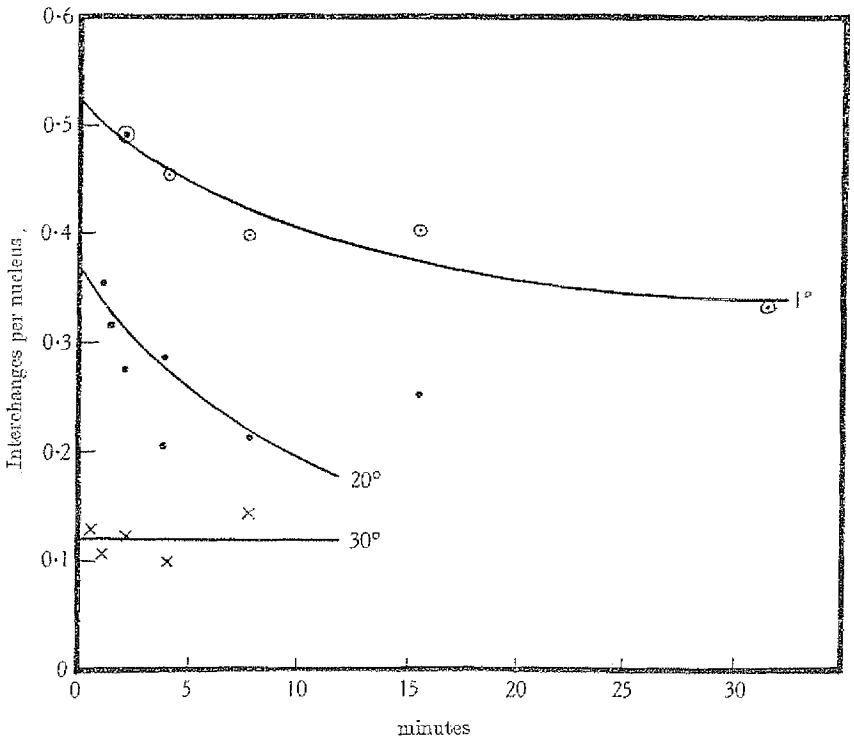

Fig. 3. c/c interchanges per nucleus produced by 150 r. at various temperatures and exposure times.

The experimental fact, illustrated in Figs. 3 and 4, that (at 1 and 20°) the yield of interchanges produced by a given dose diminishes with increase of the exposure time must mean that two breaks in a given position and produced at a time interval t have a smaller probability of exchanging than the same two breaks at the same positions would have if produced simultaneously. We may designate by $f(t)$ the factor by which the probability is reduced. With increase of t, $f(t)$ diminishes from the value unity at $t = 0$.

Consider two breaks independently produced by an irradiation at uniform intensity which extends over a time T. Each of them may occur with equal probability anywhere in the time T, and it is readily shown that the probability of the two breaks being separated by an interval between t and $t+dt$ is $2\,(1-t/T)\,(dt/T)$. The ratio G of the

number of exchanges produced by the given dose spread over time T to the number which would be produced by the same dose given in a very short time is evidently

$$G=\int_{t=0}^{T} 2\,(1-t/T)\,(dt/T)\,f(t)=2\int_{x=0}^{1} f(Tx)\,(1-x)\,dx. \qquad (1)$$

To evaluate G we must assume some plausible form for the function $f(t)$. The simplest function fulfilling the conditions of being unity at $t=0$ and diminishing gradually with increase of t is $f(t)=e^{-t/\tau}$. With this form of $f(t)$ we obtain

$$G=2\,(\tau/T)^2\,\{T/\tau-1+e^{-T/\tau}\}. \qquad (2)$$

Formula (2) has been given in an earlier paper (Lea & Catcheside, 1942), but the derivation now given may be preferred to that given earlier. A graph from which the value of G may be read off for any value of T/τ is given in the 1942 paper. In Fig. 4 we show (as curve I) a plot of the G function taking $f(t)=e^{-t/\tau}$ with $\tau=3$ min. It is seen to

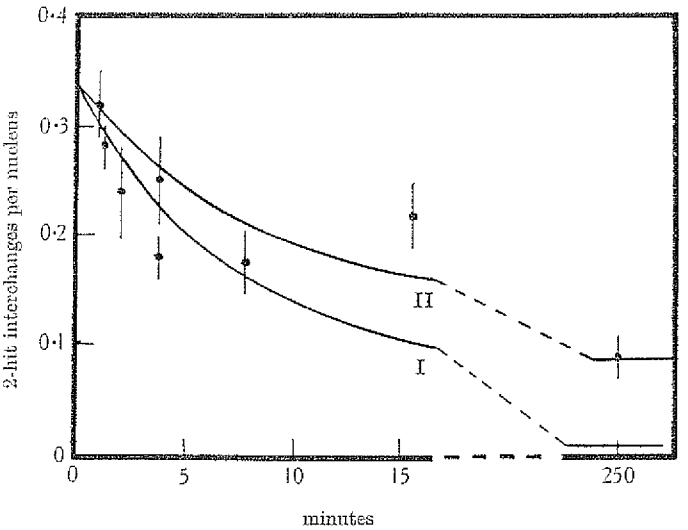

Fig. 4. Diminution of yield of 2-hit interchanges with increase of duration of exposure at 20°, with constant dose of 150 r. (points experimental, curves theoretical).

represent the experimental results satisfactorily as regards the first six points, but to descend too rapidly at long exposure times. It was at first our expectation that the persistence of an appreciable yield of interchanges in experiments in which the irradiation extended over several hours by the use of low intensities might be explained as 1-hit interchanges, but the yield of 1-hit interchanges only accounts for one-third of the total number of interchanges produced by 150 r. administered in 251 min. (see Fig. 2A) and has already been subtracted from the experimental points plotted in Fig. 4.

We are forced to the conclusion, therefore, that $f(t)$ diminishes at large values of t more slowly than is represented by the function $e^{-t/\tau}$, and a more complicated expression, e.g. $f(t)=a_1e^{-t/\tau_1}+a_2e^{-t/\tau_2}$ with $a_1+a_2=1$ will be necessary. As illustration we show as curve II in Fig. 4 the theoretical yield calculated with $a_1=0{\cdot}75$, $a_2=0{\cdot}25$, $\tau_1=3$ min., τ_2 very long. The experimental data are not adequate to determine τ_2; one can, however, assert that it is of the order of hours, not minutes.

Biologically, the separation of $f(t)$ into a short-term component (τ_1) and a long-term component (τ_2) is perhaps explicable as follows. If the two breakage ends of a newly formed break do not restitute within a few minutes, it is because they have separated outside the range of their mutual attraction, and it may be a long time before more or less accidental movements bring them within the range of attraction again.

In Table 2 we show the yields of interchanges at short exposure time, obtained by extrapolation of the yields given in the last column of Table 1 (and plotted in Fig. 3). There is a marked diminution of the yield of interchanges with increase of temperature, especially between 20 and 30°. Since we do not expect the number of chromatid breaks primarily produced to depend on the temperature, the marked fall of the yield of interchanges with rise of temperature is to be attributed to the probability of two breaks exchanging being smaller at a higher temperature than for the same two breaks at a lower temperature. Such a result could follow from the rate of restitution being increased by raising the temperature, so that less time was available for two breaks to find each other before one or both restituted. In any event, we can represent the experimental result that the yield of interchanges falls off with rise of temperature by saying that the probability $H(x)$ of exchange between two breaks separated by a distance x at the moment of their formation diminishes more rapidly with increase of x at higher temperatures than at lower temperatures.

If the reduced yield of interchanges with rise of temperature is taken to indicate that the time required for restitution diminishes with rise of temperature, then this should show up in experiments on the diminution of the yield of interchanges with increased duration of exposure time at constant dose. Comparison of the results at 1 and 20° in Fig. 3 does suggest that the yield of interchanges falls off with increasing duration of exposure somewhat more rapidly at 20 than at 1°. At 30°, however, we obtain the unexpected result that the yield of interchanges appears not to vary systematically with change of exposure time between 0·5 and 8 min. The explanation (if not experimental error) may be that the proportion of 1-hit interchanges is relatively higher at 30° than at lower temperatures. A somewhat higher proportion would in fact be expected, for reasons which will be made clear in § 6. However, the explanation remains uncertain until experiments are available directly to determine the proportion of 1-hit interchanges at 30°.

Marinelli, Nebel, Giles & Charles (1942) have also advanced a formula for expressing the yield of interchanges as a function of dose and exposure time. This is based on one developed by Swann & del Rosario (1931) for describing the lethal action of radiations on cells when recovery may occur between consecutive 'hits', and the premises do not seem appropriate in the present problem. Their formula implies that no dose, however high, can produce more than an average of 1 exchange per chromosome, or 1·5 interstitial deletions (*minutes*) per chromosome, conclusions which are unplausible and the second of which, at any rate, is known to be untrue (Newcombe, 1942, obtained an average of five interstitial deletions per chromosome by the use of the large dose of 3840 r.). Their attempt to apply a formula of Lea (1938) is similarly subject to the objection that the formula was not designed for interpreting experiments on chromosome structural changes, and its premises are inappropriate for this purpose.

Our experimental results on the manner in which the yield of interchanges diminishes with rise of temperature are in general agreement with those of Sax & Enzmann (1939).

4. Isochromatid breaks

Fig. 5 shows the yield of isochromatid breaks per cell plotted against dose for irradiation at room temperature. The most complete set of data is that of Sax (1940). His experiments, however, give a yield which is consistently lower than that obtained by ourselves, by Thoday (1942), and by Giles (1943). The discrepancy is presumably either due to a difference in dosimetry, or to a difference in sensitivity of the species of *Tradescantia* used, or to Sax's room temperature being higher than that of the other authors. Giles (1943) suggests that Sax's doses are over-estimates. We have accordingly multiplied them by 0·7, which factor brings Sax's results into accord with our own.

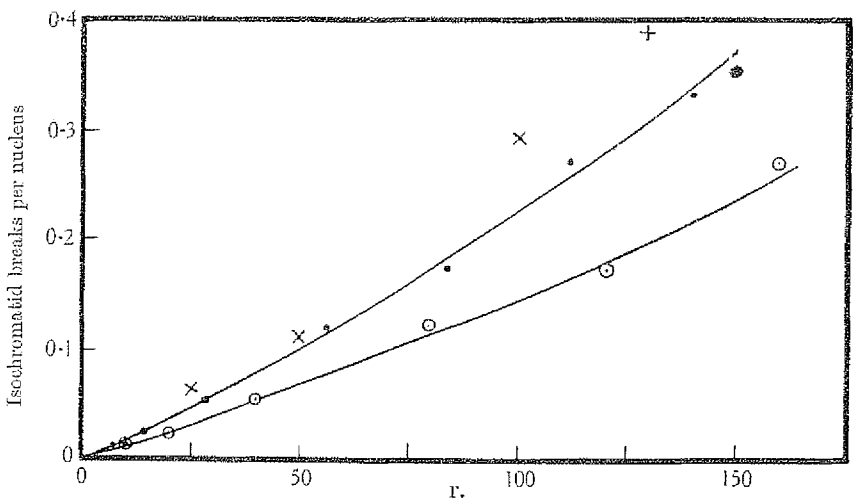

Fig. 5. Yield of isochromatid breaks as a function of dose at room temperature. ● Catcheside, Lea & Thoday (1945); × Thoday (1942); + Giles (1943); ⊙ Sax (1940), uncorrected; • Sax (1940), dose multiplied by 0·7.

The yield of isochromatid breaks increases slightly more rapidly than the first power of the dose, and the χ^2 test indicates a considerably better fit ($P=0{\cdot}6$) when the data of Sax are fitted by the equation $y=\alpha x+\beta x^2$ than when fitted by a linear equation ($P=0{\cdot}07$), despite the loss of one degree of freedom. We therefore fit Sax's data by the formula, yield of isochromatid breaks per nucleus at dose x roentgens $=\alpha x+\beta x^2$. The values of α and β (Sax's doses having been multiplied by 0·7 as described) are

$$\alpha=(0{\cdot}177\pm0{\cdot}017)\times10^{-2} \text{ per nucleus per r.},$$

$$\beta=(0{\cdot}48\pm0{\cdot}16)\times10^{-5} \text{ per nucleus per r.}^2$$

Thus at 150 r. there are $0{\cdot}265\pm0{\cdot}026$ 1-hit isochromatid breaks and $0{\cdot}108\pm0{\cdot}036$ 2-hit isochromatid breaks per nucleus, 0·373 in all.

A 2-hit isochromatid break is simply an asymmetrical exchange between two independently produced breaks which happen to lie at about the same locus in sister chromatids. The yield of 2-hit isochromatid breaks produced by a given dose presumably depends on the duration of the exposure in much the same way as does the yield of 2-hit exchanges. It should be possible, therefore, in principle, to determine the proportions of 1-hit and 2-hit isochromatid breaks at a given dose by analysing the yield into a constant component and a component diminishing with increase of exposure time. We have attempted to analyse in this way the yields of isochromatid breaks produced at 20°

by 150 r. which are set out in Table 1, and which do suggest some reduction in yield with increase of exposure time. The yields were fitted by the least squares method to the formula yield $= A + BG$, where G diminishes with increasing exposure time in the manner of curve II of Fig. 4. In this way we found that the number of isochromatid breaks produced by 150 r. in a short exposure could be analysed into 1-hit, $0{\cdot}278 \pm 0{\cdot}08$; 2-hit, $0{\cdot}095 \pm 0{\cdot}11$ isochromatid breaks per nucleus. This separation of the total yield of isochromatid breaks into 1-hit and 2-hit components is consistent with that made by analysing Sax's dose curve, but the precision is too low for the result to be of value. We shall rely therefore on the analysis of the dose curve.

5. THE DISTANCE APART AT THE MOMENT OF PRODUCTION OF BREAKS WHICH EXCHANGE

It is possible to deduce a relation between the frequencies of 2-hit isochromatid breaks and of 2-hit interchanges. The deduction depends on regarding a 2-hit isochromatid break as an exchange in which the breaks happen to lie in sister chromatids, and supposes that the probability of exchange occurring between two breaks having a given separation at the moment of production is the same whether the breaks are in sister chromatids or in separate chromosomes. Following our 1942 paper, we suppose $H(x)$ to be the probability that exchange shall occur between two breaks initially separated by a distance x, and write

$$\int_0^\infty H(x)\,dx = h_1 \quad \text{and} \quad 3\int_0^\infty x^2 H(x)\,dx = h_2{}^3.$$

Denoting by ξD the total number of chromatid breaks primarily produced by dose D, and by cD the number of 1-hit isochromatid breaks scored, $(\xi - 2c)\,D$ is the number of breaks which may be considered available for taking part in exchanges and 2-hit isochromatid breaks. These breaks are distributed at random along a length $2L$ of chromatid thread (L being the haploid length of chromosome thread). The probability that an independent break shall occur within a distance x to $x+dx$ or $-x$ to $-x-dx$ of a specified break, and in the sister chromatid, and that exchange shall occur, is therefore $(\xi - 2c)\,(D/L)\,H(x)\,dx$ which, when integrated over all x becomes $(\xi - 2c)\,(h_1/L)\,D$. To obtain the number of 2-hit isochromatid breaks we multiply by the number of breaks, $(\xi - 2c)\,D$, divide by 2 (since an isochromatid break involves 2 breaks), and again by 2 (since only asymmetrical exchanges are scored as isochromatid breaks). The yield of 2-hit isochromatid breaks produced by dose D is thus

$$\frac{(\xi - 2c)^2 h_1 D^2}{4L}. \tag{3}$$

The formula for the yield of 2-hit exchanges has already been given in our 1942 paper (equation (11). Note that in the 1942 paper we used the term *interchange* to cover both interchanges and intrachanges, i.e. in the sense in which we now use the term *exchange*). Multiplying it by $\frac{5}{6}$ we obtain for the yield of 2-hit c/c interchanges (excluding intrachanges)

$$\frac{5}{6}\,\frac{(\xi - 2c)^2 h_2{}^3 D^2}{2R^3}, \tag{4}$$

By dividing equations (3) and (4) we can eliminate $(\xi-2c)^2D^2$ and obtain

$$\frac{\text{Yield of 2-hit } c/c \text{ interchanges}}{\text{Yield of 2-hit isochromatid breaks}}=\frac{5h_2{}^3L}{3h_1R^3}=\frac{10h^2L}{R^3}, \qquad (5)$$

if, as in our 1942 paper, we take $H(x)=e^{-x/h}$, so that $h_1=h$, $h_2{}^3=6h^3$ and $h_2{}^3/h_1=6h^2$.

Since the yields of 2-hit interchanges produced by a given dose, and presumably also of 2-hit isochromatid breaks, depend on the exposure time, the yields to be inserted into equation (5) should be obtained from experiments using identical exposure times. Sax (1940) does not state the duration of the exposure in his experiment, but it was constant and appears to have been about 1 min.; the yield of 2-hit isochromatid breaks, as shown above, was $(0{\cdot}48\pm0{\cdot}16)\times10^{-5}$ per nucleus per r.[2] We therefore take the frequency of 2-hit interchanges from the part of Table 3 which refers to a 1·2 min. exposure, viz. $1{\cdot}306\times10^{-5}$ per nucleus per r.[2] Inserting these yields in equation (5) we deduce $h=0{\cdot}35\mu$.

Instead of combining equations (3) and (4) and so obtaining an estimate of h not requiring a knowledge of $(\xi-2c)$, we can make use of the value $\xi=0{\cdot}09$, whence $(\xi-2c)=0{\cdot}085$, arrived at in Paper I, and use equations (3) and (4) to derive separate estimates of h. Thus equation (4) gives the yield of 2-hit c/c interchanges produced by a dose D administered in a short time. The yield for a dose administered in 1·2 min. was $1{\cdot}306\times10^{-5}$ per nucleus per r.[2], which when corrected by the G factor with $\tau=3$ min. gives $1{\cdot}49\times10^{-5}$ per nucleus per r.[2] for a dose given in a very short time. Substituting in equation (4) we obtain $h_2=1{\cdot}02\mu$, whence $h=0{\cdot}56\mu$.

Equation (3) gives the yield of 2-hit isochromatid breaks produced by a dose D administered in a very short time. The analysis of Sax's dose curve gave $(0{\cdot}48\pm0{\cdot}16)\times10^{-5}$ 2-hit isochromatid breaks per nucleus per r.[2] Taking the exposure time in this experiment to have been 1 min., and $\tau=3$ min., we obtain $0{\cdot}53\times10^{-5}$ per nucleus per r.[2] for very short exposures. Substituting in equation (3) we obtain $h=h_1=1{\cdot}4\pm0{\cdot}5\mu$.

The estimates of h which make use of the yield of 2-hit isochromatid breaks are subject to considerable uncertainty in view of the standard deviation of 30% attaching to this yield. In addition to this random error they are liable to a systematic error. The calculation was made on the basis that a sphere of radius h centred at a given point on a chromosome intersected a length $2h$ of that chromosome. The length will, however, exceed $2h$ if the chromosome thread cannot (at 24 hr. before metaphase) be considered straight for distances of the order of one or two microns. If error on this account exists, it will make the figure $0{\cdot}35\mu$ an underestimate and the figure $1{\cdot}4\mu$ an overestimate.

Taking the three estimates into account ($0{\cdot}35\mu$; $0{\cdot}56\mu$; $1{\cdot}4\mu$) we conclude that h is of the order of 1μ, in agreement with our 1942 paper where the value $h=0{\cdot}83\mu$ was arrived at from a discussion of neutron induced exchanges.

The following calculation, while it does not lead to an estimate of h, gives some information about the function $H(x)$. When tissue is irradiated by X-rays, energy is dissipated in it by electrons (photoelectrons and recoil electrons), and it is the passage of an electron through a chromatid which causes a break. The probability of an electron breaking a chromatid through which it passes is believed (Lea & Catcheside, 1942; Catcheside & Lea, 1943) to approach unity only towards the end of the path of the electron, since the number of ionizations per micron path it produces is much greater in the 'tail' than it is earlier in the electron path. Now with X-rays of the range of wavelengths used in these experiments, an average of about four photoelectrons or recoil

electrons conclude their paths in the nucleus of radius 6μ per r. of X-rays given to the nucleus. In addition some δ-rays (short electron tracks branching off the main electron path) are produced which will increase the total number of electron 'tails' in the nucleus to about 5 per r.

Now $\xi - 2c = 0{\cdot}085$ chromatid breaks other than isochromatid breaks are primarily produced per nucleus per r. Thus the mean number of primary breaks produced per electron is $m = 0{\cdot}017$. The proportion of electrons which produce two (or more) breaks will, assuming the Poisson formula to be applicable, be $1 - e^{-m}(1+m) = 1{\cdot}43 \times 10^{-4}$, and the number of such instances will be $5 \times 1{\cdot}43 \times 10^{-4} = 7{\cdot}15 \times 10^{-4}$ per nucleus per r. Now the number of 1-hit *c/c* interchanges was found in § 3 to be $2{\cdot}3 \times 10^{-4}$ per nucleus per r., and multiplying by $\frac{6}{5}$ to allow for 1-hit *c/c* intrachanges we deduce that $2{\cdot}8 \times 10^{-4}$ 1-hit *c/c* exchanges are produced per cell per r. Comparing this with the figure $7{\cdot}15 \times 10^{-4}$ just obtained, we conclude that exchange occurs in a fraction 0·4 of the instances in which a single electron produces two primary breaks. In other words, the average value of $H(x)$ for two breaks produced by the same electron track is 0·4.

The importance of this calculation lies in the following consideration. We can be fairly confident that the probability of two breaks exchanging decreases with increase of their separation (x) at the moment of formation, so that $H(x)$ diminishes with increase of x. In making calculations we have supposed $H(x)$ to take the form $e^{-x/h}$, which implies that the probability of exchange approaches unity for two breaks which are initially very close together. It might, however, have been argued that there is no justification for this assumption, and that even for breaks very close together, restitution is much more probable than exchange. The fact that we have shown $H(x)$ to average 0·4 for two breaks produced by the same electron means that the limiting value of $H(x)$ for two breaks very close together exceeds 0·4.

6. THE DEPENDENCE OF THE YIELD OF ABERRATIONS ON THE TEMPERATURE

It is generally believed that the primary effects of radiation are independent of the temperature, and this may be considered borne out by the fact that the yield of chromatid breaks, the type of aberration most directly derived from the primary breakage process, is the type least dependent upon temperature. Interchanges involve the exchange of breakage ends, and the marked dependence of their yield upon temperature is interpreted as an effect of temperature upon the probability of exchange, not on the frequency of production of the primary chromatid breaks. This was demonstrated in a strikingly direct manner by Sax & Enzmann (1939) who found that change of temperature immediately *after* irradiation caused a change in the yield in the expected direction.

A reduction in the yield of interchanges with rise of temperature without a reduction in the number of chromatid breaks primarily produced means, in our terminology, that the probability of exchange $H(x)$ diminishes more rapidly with increase of x at high temperatures than at low. In view of the fact that the yield of 2-hit interchanges depends upon $h_2{}^3$ (equation (4)), a fairly small reduction in the value of h in the formula $H(x) = e^{-x/h}$ will suffice to explain the considerable reduction in the yield of 2-hit interchanges between 20 and 30°. The reduction in the number of 1-hit interchanges will be smaller since their yield is proportional to the first power, not the cube, of h (Lea & Catcheside, 1942, equation (11)), so that 1-hit interchanges will be relatively more important at 30 than

at 20°, as already suggested in view of the slight dependence upon exposure time of the yield of interchanges produced by a constant dose at 30° (see Fig. 3).

A reduction of the yield of isochromatid breaks with rise of temperature is perhaps to be explained as due to the sister chromatids being separated somewhat by the rise of temperature, which will reduce the probability that an ionizing particle which passes through one chromatid shall also pass through the other. It is perhaps a weakness of this explanation that it can only describe as coincidental the fact that the yields of isochromatid breaks and interchanges vary with temperature in closely parallel manner (see Table 2).*

7. Comparison of X-rays and γ-rays

Table 1 of Paper I enables the yield of *c/c* interchanges produced by X-rays and γ-rays at 20° to be compared at two exposure times. As shown in Table IV below increase of exposure time reduces the yield of interchanges produced by γ-rays in the same proportion as happens with X-rays. The yields are, however, a little less with γ-rays than with X-rays for a given dose and exposure time.

Table 4. *Comparison of yields of c/c interchanges produced by 150 r. of X-rays and γ-rays at* 20°

Radiation	Exposure min.	*c/c* interchanges per nucleus	Ratio of yields $\left(\frac{\text{long exposure}}{\text{short exposure}}\right)$		Ratio of yields $\left(\frac{\gamma\text{-ray}}{\text{X-ray}}\right)$	
X	251	0·125±0·012	X-ray	0·612		
γ	248	0·096±0·009			Long exposure	0·768±0·10
X	3·83	0·204±0·013	γ-ray	0·608		
γ	3·92	0·158±0·009			Short exposure	0·773±0·06
					Mean	0·77 ±0·05

8. Summary

1. The yield of chromatid breaks is proportional to dose, independent of exposure time at a given dose, and only slightly dependent, between 1 and 30°, upon the temperature at which the irradiation is made.

2. The yield of isochromatid breaks increases slightly more rapidly than the first power of the dose, possibly decreases slightly with increase of exposure time at a given dose, and diminishes markedly with increase of irradiation temperature especially between 20 and 30°. Most of the isochromatid breaks are 1-hit, i.e. produced by a single ionizing particle which traverses both sister chromatids, but a small proportion are 2-hit, i.e. produced by separate ionizing particles.

3. The yield of interchanges produced by X-rays increases slightly less rapidly than the square of the dose when the doses are given in a constant time. The yield produced by a given dose diminishes with increase of exposure time at 1 and 20°. The yield diminishes with rise of temperature as with isochromatid breaks. Interchanges produced by X-rays are mainly 2-hit, but a small proportion are 1-hit, and this proportion is relatively more important when long exposure times are used.

* In our experiments, the temperature treatment was confined to a period extending from 15 min. before the commencement of irradiation to 1 hr. after irradiation ended. Thereafter all series were kept at approximately 20° C. It is therefore improbable that changes in the rate of mitotic development, such as Darlington & La Cour (1945) find are caused by temperature variation, will explain our temperature effects. However, the general question of the effect of radiation temperature and intensity upon the 'drift' with time of the aberration yield is under investigation.

4. The separation at the moment of formation of breaks which exchange is of the order of 1μ. Most of the exchanges occur within a few minutes of irradiation, but a few take much longer to exchange.

5. For the same dose in roentgens, γ-rays produce slightly fewer interchanges than do X-rays.

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