CHROMATID ABERRATIONS INDUCED BY GAMMA IRRADIATION. I. THE STRUCTURE AND FREQUENCY OF CHROMATID INTERCHANGES IN DIPLOID AND TETRAPLOID CELLS OF VICIA FABA

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Received July 25, 1960

IN recent years a considerable amount of effort has been devoted to the study of the aberrations of chromosome structure induced by ionizing radiations, particularly with reference to quantitative differences due to dose, dose-rate and other controllable variables (see reviews by CATCHESIDE 1948; GILES 1954; and LEA 1955). In such experiments, aberrations involving an exchange of chromatids or parts of chromatids are conveniently classified into two groups, the intrachanges, i.e., those involving exchange within, or between, chromatids within a chromosome, and interchanges, i.e., where the exchange occurs between chromatids of different chromosomes (CATCHESIDE, LEA and THODAY 1946). In *Vicia faba* root-tip cells the chromatid intrachanges induced by ionizing radiation are of at least four different types, and studies on the relative frequencies of these types has led to a new interpretation of the mode of formation of certain classes of chromatid aberrations by ionizing radiation (REVELL 1959). In the present work a study was made of the relative frequencies of the various types of interchanges in diploid roots and in diploid and tetraploid cells of roots subjected to colchicine.

EXPERIMENTAL METHODS

Seeds of *Vicia faba* (Suttons' Prolific Longpod) were germinated and the seedlings cultured in aerated, running tap water at 19°C as described previously (EVANS, NEARY and TONKINSON 1957). On the tenth day after germination the primary roots of the seedlings were irradiated with Co^{60} gamma rays.

For the two hours prior to fixation the roots were placed in nonaerated saturated aqueous solutions of *p*-dichlorobenzene (EVANS and TONKINSON 1959) and fixations of the terminal 2 cms of primary root were made, under vacuum, at intervals between 3½ and 8 hours after irradiation. Fixations were made in a solution made up of two percent osmic acid, two percent chromic acid and distilled water in the ratio (v/v) of 1:1.7:2; after 24 hours the material was thoroughly washed and allowed to bleach and macerate in a 1:6 (v/v) mixture of 40 vol. hydrogen peroxide and ammonium oxalate (saturated aqueous solution) for 20 minutes (C. E. FORD, personal communication). The roots were stained by the Feulgen procedure and the terminal 1.5 mm of each primary root was used to make one squash preparation.

The data on interchange frequency in diploid roots presented in the first part

of this paper is from four separate experiments in which radiation doses of from 51 to 147 rads were used—all the doses being given over a duration of 4½ to 5½ minutes. For the experiment with colchicined roots on the ninth day after germination the seedling roots were treated with an aerated 0.05 percent aqueous solution of colchicine for 3½ hours at 19°C (EVANS and SAVAGE 1959) then thoroughly washed and replaced in tap water at the same temperature. Twenty-five hours after the commencement of the colchicine treatment the roots were irradiated with 118 rads of Co^{60} gamma rays given in 3.8 minutes. The roots were pretreated with *p*-dichlorobenzene and fixed at four, five and six hours after irradiation, as described above. Four slides were scored at each fixation time, and on each slide 200 diploid and 150 tetraploid cells were scored for chromatid interchanges.

All the slides were coded and randomized for each experiment. Less than two percent of the total interchanges observed were rejected as being difficult to score, usually because of the complex nature of the interchange, so that observational bias from this source was negligible.

Experiments with diploid roots

Chromatid interchanges are usually classified on the basis of two characteristics. symmetry and completeness. If the exchange results in the production of a dicentric chromatid and an acentric fragment, or if it is of a type which if completely fulfilled would give rise to a dicentric chromatid, then it is termed asymmetrical (A), as opposed to the symmetrical situation (S) where each chromatid has one centromere. If the exchange is not completely fulfilled, so that of the four broken ends which are assumed to be produced only two undergo union, then the exchange is termed incomplete (I) as opposed to the complete (C) condition where all ends undergo union.

In some of the early experiments carried out in this laboratory it was found that the asymmetrical interchanges occurred more frequently than the symmetrical types. Such an excess of A type over S type interchanges has been noted by a number of authors (Table 1), although LEA (1955), summarizing the data available up until 1946, stated that although in some experiments there was a significant excess of A over S types "it is doubtful if the reliability of the distinction between symmetrical and asymmetrical interchanges is sufficient to make such a difference certain" and suggested that S and A type interchanges were approximately equally frequent.

The separation of interchanges into two categories on the basis of symmetry is rather an indirect means of classification as the symmetry of an interchange is seen to be dependent upon two factors; (1) the polarity of the chromosomes involved, and (2) the manner in which the chromatid parts are exchanged. Chromosome polarity is probably a consequence of the anaphase movement of the chromosomes at mitosis, as the centromeric regions of the chromosomes pass first to the poles so that the centromeres lie closely grouped together at the later stages of mitosis. If little relative displacement occurs following the uncoiling and elongation of the chromosomes and during the succeeding interphase, then

TABLE 1

Relative frequencies of asymmetrical and symmetrical interchanges found by various authors

Material	No. of interchanges scored		Reference
	Symmetrical	Asymmetrical	
Tradescantia, pollen grain mitosis	26	66	SAX and MATHER (1939)
	182	402	SAX (1940)
	1613	1424	CATCHESIDE, LEA and THODAY (1946)
Tradescantia, pollen tube mitosis	49	46	NEWCOMBE (1942)
	15	70	SWANSON (1942)
	53	60	SWANSON (1942)
	12	9	CATCHESIDE and LEA (1943)
Chortophaga neuroblasts	5	9	CARLSON (1941)
Vicia faba root tips			THODAY (1948)
X rays	171	182	
α rays	151	190	

at the early prophase stage of the following mitosis the chromosomes should still show polarity. Evidence that no extensive chromosome movement occurs during interphase is given by the classical observations of BOVERI (1909) on Ascaris embryos and BELAR (1929) on the staminal hair cells of Tradescantia; these authors found that the chromosomes at prophase maintained roughly the same relative positions that they were observed in at the late stages of the previous mitosis. SAX (1940) found that the interphase chromosomes in Tradescantia microspores were strongly polarized as unequal interchanges induced by radiation were rare, and over 80 percent of the interchanges were found to involve exchange between chromosomes or chromatids at corresponding loci in respect of the distance from the centromeres. A similar conclusion is arrived at for the chromosomes in root-tip cells of *Vicia faba*. In an early experiment, interchanges were classified on the basis of symmetry and completeness and also with regard to the distance separating the centromeres, equal or roughly equal interchanges were defined as those in which the two centromeres were within a given axial distance from one another, the distance being arbitrarily defined in this instance as being about one quarter of the length of an acrocentric metaphase chromosome (about 2μ). Of 93 interchanges scored in this experiment 79 (i.e., 85 percent) were found to involve polarized chromosomes and 63 (70 percent) polarized chromosomes whose centromeres were close together, i.e., equal or roughly equal interchanges.

The second factor which determines the symmetry of an interchange relates to the actual mechanics of the exchange event itself. An exchange between chromatids of two chromosomes can occur in two ways as shown in Figure 1. The exchange can be similar to the type of exchange which occurs normally at meiosis, which we may call the X type exchange (Figure 1b), or the exchange may be of the form shown in Figure 1a which we may simply call the U type

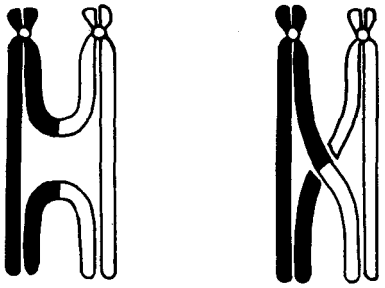

FIGURE 1.—Diagram showing two interchanges as observed at metaphase illustrating (a) the U type exchange and (b) the X type exchange.

exchange. An X type of exchange between chromatids of polarized chromosomes will yield a symmetrical interchange, whereas a U type exchange will be asymmetrical because of the formation of a dicentric chromatid.

On the three criteria of polarity of the chromosomes—(polarized, P, and non-polarized, N) type of exchange (X or U), and completeness (complete, C, and incomplete, I)—we can classify chromatid interchanges into eight morphologically distinguishable types, and the two asymmetrical incomplete types (PUI and NXI) can be further subdivided into four groups (Figure 2). The interchanges scored in four separate experiments were classified into these eight categories and the results obtained are shown in Table 2.

CATCHESIDE, LEA and THODAY (1946) showed that the relative frequency of A to S chromatid interchanges and of C to I types, was independent of dose, dose-rate, and temperature. Similarly in the present experiments there was no detectable effect of dose on symmetry and completeness. However, before going on to an analysis of the results a further factor must be borne in mind, namely the question of whether the mechanical pressure exerted on the cells during the preparation of the squash influences the degree of completeness and the orientation of the interchanges. In a few of the cells in a well squashed root-tip preparation the satellites of the two metacentric chromosomes are sometimes broken off at the nucleolar constriction, due to the mechanical pressure exerted on the slide. There also appears to be a tendency for such mechanical breakage to occur at the points where achromatic lesions or "gaps" have been induced by the radiation, and such fractures are morphologically indistinguishable from true chromatid deletions induced by the radiation. The importance of such mechanical breaks in interchange associations is not known, but it is likely that a small proportion of the complete interchanges are scored as incomplete types, due to mechanical breakage occurring at the point where the exchange took place. Mechanical pressure could also influence the orientation of an interchange in that an X type exchange could be squashed into a U type or *vice versa;* for example a PXC interchange would be scored as NUC if on squashing the chromosomes were displaced in different directions or if one of the chromosomes was

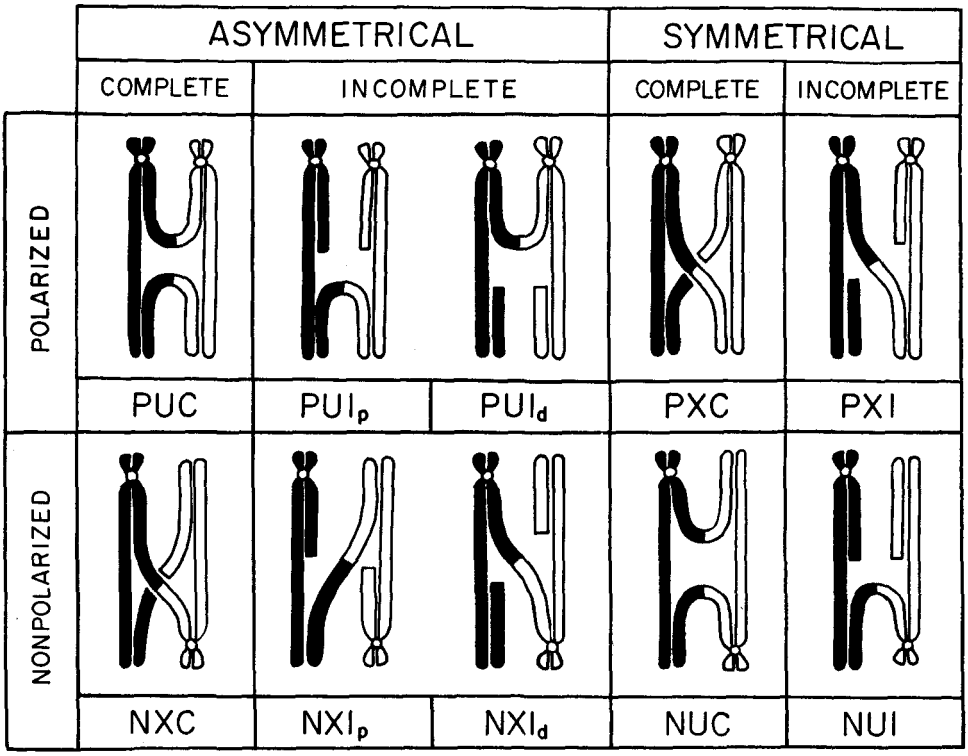

FIGURE 2.—Diagram showing the ten types of chromatid interchange P = polarized; N = nonpolarized; U and X are types of exchange; C = complete; I = incomplete; Ip = proximally incomplete; Id = distally incomplete.

TABLE 2

*Frequencies of the various interchange types in four experiments on diploid roots**

Interchange type	Asymmetrical				Symmetrical				
Expt.	PUC	PUI	NXC	NXI	NUC	NUI	PXC	PXI	Totals
1	121 (0.48)	35 (0.14)	2 (0.01)	2 (0.01)	15 (0.06)	1 (0.004)	40 (0.16)	34 (0.14)	250
2	74 (0.49)	27 (0.18)	1 (0.01)	1 (0.01)	9 (0.06)	3 (0.02)	23 (0.15)	12 (0.08)	150
3	80 (0.35)	35 (0.15)	9 (0.04)	4 (0.02)	12 (0.05)	8 (0.04)	48 (0.21)	32 (0.14)	228
4	50 (0.50)	10 (0.10)	1 (0.01)	1 (0.01)	13 (0.13)	5 (0.05)	15 (0.15)	5 (0.05)	100
Totals	325 (0.45)	107 (0.15)	13 (0.02)	8 (0.01)	49 (0.07)	17 (0.02)	126 (0.17)	83 (0.11)	728

* The figures in parentheses are the frequencies expressed as percentage values. The symbols P and N are for polarized and nonpolarized chromosomes, U and X are types of exchange, C and I are for complete and incomplete interchange types.

turned through 180°. It is likely that gross localized displacement of chromosomes due to squashing is an infrequent event, and this is evidenced by the fact that only 12 percent of the total interchanges scored were found to have nonpolarized chromosomes at metaphase (types NX and NU in Table 2), and also by the observation that the ratios PX:NU and NX:PU are dissimilar.

The results obtained in the four experiments shown in Table 2 have been subjected to analysis in order to examine the main effects—polarity, type of exchange and completeness—and to see if there were any interactions between these effects. The main effects were subjected to χ^2 or variance ratio tests and all were found to differ significantly from a hypothetical 1:1 ratio; in addition there was a significant excess of asymmetrical over symmetrical interchange types (Table 3). Interactions were examined by fixing the main effects to be as observed, and the analyses were performed using partition of χ^2 (LANCASTER 1951) and the log likelihood ratio test (KULLBACK 1959). Both analyses showed that there was no effect of polarity on completeness (PC) or of polarity on the type of exchange (PU). However, the interaction between completeness and type of exchange (CU) was found to be significant at the five percent level: further examination showed that this interaction was largely concentrated in experiment 1, which was the first experiment in which interchange aberrations were scored, and an analysis of the data excluding the first experiment revealed no significant interactions.

The results thus indicate that a large degree of chromosome polarity is present in the interphase nucleus and that chromosome polarity does not influence the type of exchange. Of the two possible types of exchange that can occur between chromatids the chiasma or X type exchange occurs less frequently than the U type; because of this, coupled with the high frequency of polarized chromosomes, asymmetrical interchanges are more frequent than symmetrical types.

TABLE 3

Analyses of main effects for agreement with a hypothetical 1:1 ratio between the two possible classes for each effect

Effect	Observed ratio	χ^2	P
Polarity			
P:N	0.88 : 0.12	442	< 0.001
Exchange			
U:X	0.68 : 0.32	*	0.5–0.01
Completeness			
C:I	0.70 : 0.30	122	< 0.001
Symmetry			
A:S	0.62 : 0.38	44	< 0.001

* Because of heterogeneity between experiments for this effect the analysis was performed using a variance ratio test (F_1 and $F_3 = 20.7$).

The experiment with roots treated with colchicine

Following upon the finding of a high degree of polarity of the chromosomes and the inference that no gross chromosome movement occurred during interphase, an experiment using a colchicine technique was set up to give further information on these points and also on the effect of polyploidy on the frequency of interchange aberrations. The technical details of the colchicine experiment have been described under the heading of Experimental Methods and the rationale behind the experiment was as follows. Roots were subjected to colchicine for 3½ hours and then returned to water so that cells in the early stages of mitosis and the later stages of interphase at the time of cholchicine treatment would not pass through a normal anaphase stage and would become polyploid as a result of spindle inhibition, while the cells in the earlier stages of interphase and in the postmetaphase stages of mitosis at the commencement of treatment would remain diploid. In this way, following the colchicine treatment, the meristematic regions of the roots would consist of a mixture of diploid and tetraploid cells and as the mitotic cycle in these root tip cells at 19°C takes about 18 to 24 hrs, some of these tetraploid cells would be in mitosis about a day after the colchicine treatment (EVANS, NEARY and TONKINSON 1957, 1959). Due to the natural variation in the rate of development of cells through the mitotic cycle, at the time of irradiation (21½ hours after completion of the colchicine treatment) diploid and tetraploid cells in approximately the same stage in development were being irradiated and the chromatid aberrations in such cells could be observed at metaphase in roots which were fixed between four and six hours after irradiation.

The results obtained from the colchicine experiment are described under three separate headings.

1. *The effect of colchicine treatment on chromosome polarity:* The frequencies of the various types of interchange were determined from scores obtained from 2,400 diploid and 1,800 tetraploid cells. The interchanges were categorized as described in the first part of this paper, and the results obtained are given in Table 4.

It was anticipated that in the tetraploid cells, which would have passed through two interphase periods in the absence of the polarizing influence of an intermediary anaphase, there might be a reduction in the amount of polarity and consequently in the frequency of A type interchanges. The results in Table 4 show that there is in fact little difference between the frequency of polarized interchanges in diploid and tetraploid cells (78 percent and 84 percent, respectively) and that the frequencies are similar to those obtained in the four experiments on diploid roots reported in the first part of this paper. The results thus suggest that there is no large scale chromosome movement during "c"-mitosis and that a high degree of chromosome polarity might still be in evidence over a number of mitotic cycles even in the absence of mitotic spindles.

To test for possible differences in the relative frequencies between diploid and

TABLE 4

*Frequencies of the various interchange types in diploid and tetraploid cells in roots subjected to colchicine**

Interchange type	Asymmetrical				Symmetrical				
Ploidy	PUC	PUI	NXC	NXI	NUC	NUI	PXC	PXI	Totals
2n	250 (0.33)	132 (0.17)	55 (0.07)	18 (0.02)	78 (0.10)	19 (0.03)	164 (0.22)	48 (0.06)	764
4n	361 (0.33)	184 (0.17)	59 (0.05)	24 (0.02)	79 (0.07)	27 (0.02)	265 (0.24)	111 (0.10)	1,110
Totals	611 (0.33)	316 (0.17)	114 (0.06)	42 (0.02)	157 (0.08)	46 (0.03)	429 (0.23)	159 (0.08)	1,874

* The figures in parentheses are the frequencies expressed as percentage values. The symbols P and N are for polarized and nonpolarized chromosomes, U and X are types of exchange, C and I are for complete and incomplete interchange types.

tetraploid cells the data in Table 4 were analyzed employing the two methods used for the data on diploid roots—the log likelihood ratio test (KULLBACK 1959) and partition of x^2 (LANCASTER 1951)—and a significant difference between diploid and tetraploid data was suggested ($P_7 = 0.02$). On further inspection it was found that this was due to interactions in the diploid data which were mainly the result of the relatively lower frequency of PXI type interchanges; omission of the PXI class from the analysis resulted in homogeneity and no significant difference between diploid and tetraploid cells, ($P_6 = > 0.2$). Averaging the total data gives the frequency of polarized interchanges as 81 percent, U type exchanges 61 percent, completeness 70 percent, and asymmetrical interchanges as 58 percent. These values are similar to those obtained from the experiments on diploid roots (Table 3).

2. *The frequency of interchange between chromatids of homologous chromosomes in diploid and tetraploid cells:* The chromosome complement of diploid cells of *V. faba* is made up of one pair of easily distinguishable metacentric, or M, chromosomes and five pairs of acrocentric chromosomes, the S chromosomes (Figure 3). The two arms of an M chromosome are dissimilar both in length and in morphology and are easily distinguished, the shorter arm (M_2, as opposed to the long arm M_1) having a secondary constriction at the nucleolar organizing region. Of the five pairs of S chromosomes three (Sa, Sd and Se) are easily separated on the basis of the length of the long and short arms, and the remaining two chromosomes, Sb and Sc are very similar. In the present paper the S chromosomes will generally be treated as one group, and details of the frequency of interchange between the various S chromosomes will be presented in a separate paper.

A simple estimate of the frequency of interchange between homologous chromosomes or chromosome arms can be obtained by determining the frequency of M_1 to M_1, M_2 to M_2, and S to S interchanges. Now in cells which have been allowed to proceed through a c-mitosis the chromatids of a given chromo-

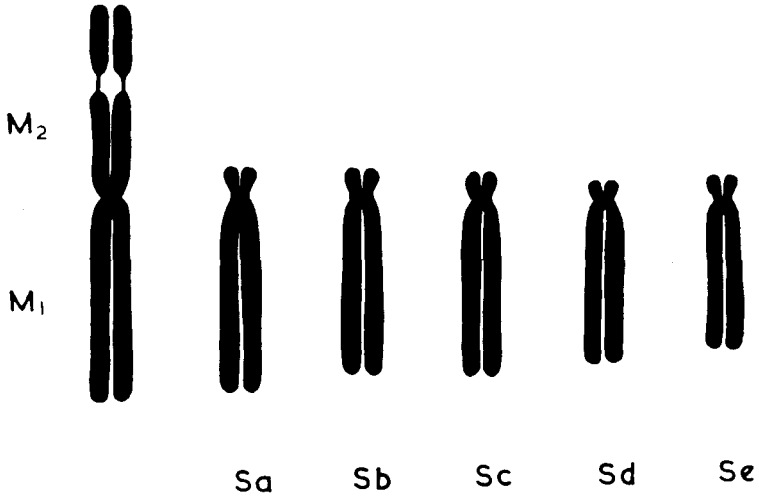

FIGURE 3.—Diagrammatic representation of the haploid chromosome set of *Vicia faba* (Sutton's Prolific Longpod).

TABLE 5

*The frequencies of interchange between similar and different chromosomes in diploid and tetraploid cells**

Fixation time (hrs)	Interchange type / Ploidy	S to S	M to S		M to M			Total interchanges
			M_1 to S	M_2 to S	M_1 to M_1	M_1 to M_2	M_2 to M_2	
4		61	23	9	2	3	2	100
5	2n	57	21	16	2	3	1	100
6		64	12	20	1	2	1	100
Total		182	56	45	5	8	4	300
		(0.607)	(0.187)	(0.150)	(0.017)	(0.027)	(0.013)	
4		61	17	10	6	4	2	100
5	4n	67	14	11	2	3	3	100
6		64	13	15	3	3	2	100
Total		192	44	36	11	10	7	300
		(0.640)	(0.147)	(0.120)	(0.037)	(0.033)	(0.023)	

* Percentage values are given in parentheses.

some will not be separated to opposite poles of the cell because of the absence of a spindle: thus, if there is little chromosome movement following the separation of the chromatids and throughout the following interphase, such cells, which will appear as tetraploids at the next mitosis, should show a higher frequency of interchange between homologous chromosomes than their diploid counterparts. The possibility of a difference in the relative frequency of interchange between homologous chromosomes in diploid and tetraploid cells was investigated, and 300 interchanges in diploid cells and 300 in tetraploid cells were scored and classified into the six possible groups as shown in Table 5.

The results in Table 5 are interesting in two respects. Firstly the relative frequency of the interchange classes S to S, M_1 to M_1 and M_2 to M_2 is seen to be increased in the tetraploid cells by roughly six percent. Now the small increase in S to S type interchanges is not very revealing as we have no information on how many of the S to S type interchanges have taken place between truly homologous chromosomes. On the other hand, although there are relatively fewer interchanges involving M to M chromosome, exchanges between homologous chromosome arms have been distinguished and are seen to be twice as frequent in tetraploid cells as opposed to the diploid cells. Although a small increase in the frequency of homologous interchanges is to be expected on statistical grounds (EVANS and BIGGER 1961), the observed doubling of the frequency of homologous interchanges in tetraploid cells is additional evidence indicating that little chromosome movement occurs during interphase.

The second interesting piece of information from these data concerns the question of the relation between chromosome length and the probability of a chromosome taking part in an interchange. We have measured the lengths of the metaphase chromosome in 12 cells and the average values, converted to microns, are $M_1 = 8.7$, $M_2 = 8.0$, and the five S chromosomes give a total of 36.1; the ratio, in terms of length, of the metacentric to acrocentric chromosomes is thus M:S = 1:2.16. Now if the probability of a chromatid undergoing interchange is a simple function of chromosome length, then the ratio of M:S chromosomes which have undergone exchange should conform to the ratio of 1:2.16. It may be seen from the data in Table 5 that the results do not fit this expectation ($\chi^2 = 45.4$, $P = < 0.001$), for the observed ratio in which these chromosomes take part in interchange is 1:3.44 for both diploid and tetraploid cells, i.e., the S chromosomes undergo interchange more frequently than is expected on the basis of metaphase chromosome length. The relative frequency of exchange by the arms of the M chromosome, i.e., M_1:M_2, with themselves or with the S chromosomes (but excluding the interarm M_1 to M_2 type interchanges), should on the basis of relative lengths be M_1:M_2 = 1:0.92, the observed frequency with which these chromosomes undergo exchange (data from Table 5) is 1:0.78. There is thus an apparent tendency for the M_2 chromosome arm to undergo rather less exchange than is predicted, but this does not differ significantly from expectation ($\chi^2 = 1.57$, $P = 0.2$–0.3). The data as a whole suggests that the probability of a chromatid taking part in an interchange is not a simple function of its length at metaphase; further and more detailed experimental results on this point will be reported in the second paper in this series.

3. *The effect of polyploidy on the frequency of interchange and isochromatid aberrations:* The influence of polyploidy on chromosome radiosensitivity has been the subject of study by a number of authors (SAX and SWANSON 1941; FRÖIER, GUSTAFSON and TEDIN 1942; and SMITH 1943, 1946). BISHOP (1952) found that the chromosome aberration frequency, per cell, in microspores of the tetraploid *Tradescantia virginiana* (2n = 24) was, in general, twice the frequency produced by the same radiation dose in *T. paludosa* (2n = 12). Thus the aberration frequency per chromosome was the same in the two species, although other data

obtained from cells irradiated in the interphase prior to pollen tube mitosis indicated that the tetraploid cells of *T. virginiana* were significantly more than twice as sensitive than the diploid *T. paludosa*. The difficulties of comparing aberration frequencies in diploid and tetraploid cells of different species, or of cells irradiated at different stages in development, were eliminated when CONGER and JOHNSTON (1956) scored chromosome aberration frequencies in an irradiated *Tradescantia paludosa* flower bud, which on examination was found to consist of a mixed population of haploid and diploid microspores. CONGER's results showed that for both chromosome deletions and exchanges, the aberration frequency in diploid microspores was exactly twice the frequency in haploid cells.

In the present work the production of root chimaeras containing both diploid and tetraploid cells, and the irradiation of such cells at similar stages in development along the mitotic cycle, offered an excellent opportunity for examining the influence of polyploidy on radiosensitivity in a different species and using chromatid as opposed to chromosome aberrations as a measure of effect.

The frequencies of interchanges, obtained from the data scored for the relative frequencies of the various types of interchange (Table 4), are given in Table 6. An analysis of variance on the data showed that there was no effect of fixation time on interchange frequency ($VR = 0.004$, $P_{(2,18)}, = > 0.2$), but a highly significant difference existed between the frequencies in 2n and 4n cells ($VR = 35.3$, $P_{(1,18)}, = < 0.001$). The over-all mean frequency of interchanges in 2n cells was 0.318 ± 0.06, and in 4n cells 0.617 ± 0.07; interchanges are thus seen to be twice as frequent, on a per cell basis, in the tetraploid cells (χ^2 for a 2:1 ratio of 4n:2n $= 0.46$, $P = 0.5$). Expressing the data in terms of interchanges per chromosome, and treating the arms of the metacentric chromosomes as separate chromosomes, the frequency of interchange is the same in 2n as in 4n cells and is respectively 0.0227 ± 0.0044 and 0.0220 ± 0.0026 per chromosome ('t' between chromosomes in 2n and 4n cells $= 0.13$, $P_{18} = 0.9$).

TABLE 6

Frequency of chromatid interchanges in diploid and tetraploid cells

Fixation time (hrs)	Ploidy	Total no. of cells scored	No. of normal cells	No. of cells containing one or more interchanges				Total no. of interchanges scored	Mean interchange frequency per cell
				1	2	3	4		
4	2n	800	565	225	10	..	..	245	0.31 ± 0.04
	4n	600	250	295	43	10	2	419	0.70 ± 0.04
5	2n	800	577	215	8	..	..	231	0.29 ± 0.04
	4n	600	310	240	44	5	1	347	0.58 ± 0.04
6	2n	800	530	253	16	1	..	288	0.36 ± 0.02
	4n	600	303	257	33	7	..	344	0.58 ± 0.05
Totals	2n	2,400	1,672	693	34	1	..	764	0.318 ± 0.06
	4n	1,800	863	792	120	22	3	1,110	0.617 ± 0.07

In addition to scoring for interchanges the frequency of isochromatid breaks in 2n and 4n cells was also determined, and the results are presented in Table 7. As in the case of the interchange class, isochromatid breaks are twice as frequent in 4n cells as in 2n cells (χ^2 for a 2:1 ratio of 4n to 2n = 0.18, P = 0.7). However, one interesting difference between the frequencies of isochromatid and interchange aberrations is seen from a comparison of the relative distribution of these aberrations between cells. It may be seen (Table 8) that the relative frequencies of cells containing different numbers of isochromatid aberrations gives a good fit to a Poisson distribution, whereas the distribution of interchanges between cells, both for 2n and 4n types, is not Poisson.

DISCUSSION AND CONCLUSIONS

Chromosome polarity and chromosome movement during the mitotic cycle: The results from the experiments on diploid roots on the frequencies of the various interchange types have shown that about 88 percent of the interchanges observed at metaphase have occurred between polarized chromosomes. Although

TABLE 7

Frequency of isochromatid aberrations in diploid and tetraploid cells

Ploidy	Total no. of cells scored	No. of normal cells	No. of cells containing one or more isochromatid aberrations			Total no. of isochromatid aberrations	Mean frequency of isochromatid aberration per cell
			1	2	3		
2n	200	180	19	1	. .	21	0.105 ± 0.023*
4n	200	160	34	5	1	47	0.235 ± 0.034*

* The standard errors have been determined assuming a Poisson variate.

TABLE 8

The frequencies of cells with interchanges and isochromatid breaks, and the expected frequencies assuming a Poisson distribution

Aberration	Ploidy	Distribution	Frequencies of cells with indicated no. of aberrations					χ^2
			0	1	2	3	4	
Interchange	2n	Observed	1672	693	34	1	. .	78.9**
		Expected	1746	356	88	10	. .	
	4n	Observed	863	792	120	22	3	105.7**
		Expected	971	599	185	38	7	
Isochromatid	2n	Observed	180	19	1	. .	. .	. . . .
		Expected	180	20	.	.	. .	
	4n	Observed	160	34	5	1	. .	0.63
		Expected	158	37	5	.	.	

** Significant at the 0.1 percent level.

10–20 percent of the interchanges are between chromosomes which at metaphase, have centromeres aligned in opposite directions, this does not necessarily imply that these chromosomes were not polarized in interphase. An apparent non-polarity at metaphase of chromosomes involved in an interchange could be due to the exchange occurring between overlapping distal ends of chromosomes whose centromeres are polarized to one side of the spherical interphase nucleus. We can conclude therefore that at least 80–90 percent of the chromosomes are polarized during interphase and that this polarity is probably a consequence of the grouping of centromeres following their movement along the mitotic spindle at the anaphase stage. The fact that this polarity is maintained throughout interphase implies that in spite of chromosome elongation and uncoiling at the late stages of mitosis there is relatively little change in position of chromosomes due to movement during interphase.

The above conclusions are reinforced by two types of observation made on interchange frequencies in the diploid-tetraploid root chimaeras. Firstly the finding that the relative frequency of interchange between polarized chromosomes in diploid cells was similar to that observed in tetraploid cells produced by colchicine, indicates that there is little change in the orientation and relative positions of the chromosomes during 'c'-mitosis as well as throughout two successive interphase cycles. It is of interest to note that many of the different types of movement of metaphase chromosomes, as observed in living cells using cinematographic methods (BAJER 1958), are absent or very much reduced in the absence of a mitotic spindle in 'c'-mitotic cells (MOLE-BAJER 1958).

The second important result having a bearing on the amount of chromosome movement that occurs during interphase is the observation that the irradiation in late interphase of cells which were allowed to proceed into that interphase without a true anaphase separation of chromatids, results in an appreciable increase in the frequency of interchange between homologous chromosomes. As may be seen from Table 5, the relative frequency of homologous interchange between the arms of the metacentric M chromosomes is twice as great in the tetraploid cells as in their diploid counterparts. This result suggests that there is little lateral movement of the chromosomes during interphase, and in the light of the observations on the polarity of the chromosomes it would appear that, from a mechanical point of view, the interphase chromosome is a relatively static structure.

The symmetry and completeness of interchanges and the nature of the exchange event: Interchange aberrations result in an exchange of chromatid parts between chromosomes and are in this sense similar to the normally occurring process of chiasma formation at meiosis. Beyond this, the comparison between radiation (or chemically) induced interchange and chiasma formation appears to break down. Three major points of difference are that chiasmata are formed between identical loci of homologous chromosomes, are never incomplete, and are always of the X type and are thus symmetrical, although an exception to this latter point is seen for example in the formation of a dicentric chromatid following normal X type chiasma formation within an inversion loop. In spite of these obvious differences between chiasmata and mitotic interchanges, and the fact that

they are initiated or produced by different means and in different cells, it is possible that the basic mechanics of exchange could be the same for both events, and that the asymmetry and incompleteness of an interchange may be a consequence of the relative positions of the chromatids involved with respect of the points of damage produced by the radiation and may not be *entirely* a function of the radiation itself.

The finding that at metaphase there are seen to be two types of exchange, the U and the X type, might suggest that there are indeed two different methods of exchange which can take place at the points of damage. We have shown that there is a significant excess of the U type as opposed to the X type exchange and this coupled with the high degree of chromosome polarity results in an excess of asymmetrical interchanges, but there is no interaction between the type of exchange and the polarity of the chromosomes involved. An alternative explanation for the presence of two types of exchanges at metaphase is that the exchange is of one form only and that the appearance of both U and X types at metaphase is the result of differences in the arrangement of the chromatids involved at the time when the interchange took place. We can illustrate this point as follows: if a chiasma-like, i.e. X type, exchange occurred between chromatids of two chromosomes, and at the point of exchange both chromatids were coiled, or both uncoiled, then the interchange would appear as an X type at metaphase. If however, an X type exchange occurred between a coiled and an uncoiled region of the two chromatids, then at metaphase the interchange would be of the U type (Figure 4), but the dicentric and acentric fragment should in some cases be interlocked. Such interlocking has not been observed, so that the presence of two types of exchange at metaphase is probably not a consequence of the fact that the chromosomes involved are not truly paired, but rather is an indication that the exchange process following radiation may take one of two forms.

No association was found between chromosome polarity, or type of exchange,

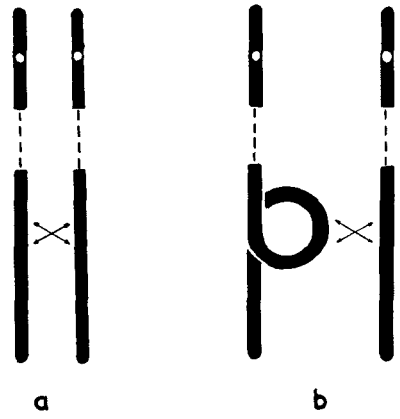

FIGURE 4.—The formation of interchanges which appear as X (a) and U (b) types at metaphase, although both are the result of X type exchanges during metaphase.

and incompleteness, although as reported by a number of authors (DARLINGTON and LA COUR 1945; CATCHESIDE, LEA and THODAY 1946) there is a higher frequency of incompleteness at the centromere, or proximal, side of the interchange. Of the 115 asymmetrical, incomplete, interchanges scored in diploid roots (PUI and NXI in Table 2) 0.57 were found to be incomplete on the proximal side of the interchange, cf. with 0.59 and 0.50 found in Tradescantia microspores by CATCHESIDE *et al.* (1946).

The amount of incompleteness is known to be influenced by the type of radiation used, for instance there may be more incompleteness with α radiation than with X or γ radiation (KOTVAL and GRAY 1947; FABERGÉ 1959), and this has led to the suggestion that incompleteness may be the result of severe damage to the chromatids at the points where the exchange will occur (LEA 1955). It is also possible that incompleteness may, in part, be a function of the distance separating the points which undergo exchange. If spatial separation is important, then in X or γ irradiated material there might be a possible association between the proportion of interchanges that are incomplete and the proportion that are two-hit although it should be noted that at present there is no evidence for correlation between dose-rate and incompleteness. In addition, it is important to point out that in squash preparations, some incompleteness may be due to mechanical breakage of a complete exchange due to the pressure used in the preparation of the slide.

The relation between chromosome length and interchange frequency: From the data in Table 5, which gives the frequency of interchanges involving S and M chromosomes, it has been shown that the frequency of interchange per chromosome does not appear to be directly proportional to the metaphase length of the chromosome. Although there are a number of instances where aberrations induced by radiation have been found to be distributed in a non random fashion along the length of the metaphase chromosome (see review by SPARROW 1951; EVANS and BIGGER 1961), the statement is frequently made, particularly with reference to radiation induced aberrations in Vicia (FORD 1949; DEUFEL 1951; REVELL 1953), that the distribution of such aberrations *between* chromosomes is in direct proportion to the metaphase length of those chromosomes. The present findings for interchange aberrations are at variance with this suggestion and following upon the present observations a detailed study of the distribution of interchange and isochromatid aberrations along the chromosomes was carried out, and the results and further discussion on this point will be presented in the second paper in this series.

The effect of polyploidy on chromatid aberration frequency: The frequency of both chromatid interchanges and isochromatid breaks in tetraploid cells was found to be twice the frequency observed in diploid cells. If primary breakage in the nucleus increases linearly with increasing dose, then it is to be expected that a doubling of the dose or of the chromosome number would both result in a doubling of the total number of breaks in the nucleus. However, as CONGER and JOHNSTON (1956) have pointed out, doubling the dose would double the fre-

quency of breakage per unit nuclear volume, but in the case of a doubled chromosome number if such an increase in chromosome number also results in a corresponding increase in nuclear volume, then the number of breaks per unit volume would remain constant.

A change in nuclear volume in tetraploid as against the diploid cells in Vicia would be of little importance with regard to intrachromosome aberrations and the finding that isochromatid aberrations are twice as frequent in 4n than in 2n cells is as might be expected. On the other hand, interchange aberrations involve the participation of two chromosomes and in Vicia, at the dose rates used in the present experiments, these aberrations are known to be predominantly two-hit and to increase as approximately the square of the dose (NEARY and EVANS 1958). There is some reason therefore for expecting that the interchange frequency in 4n cells might be four times and not twice the frequency found in 2n cells. In this respect the density of chromosomes per unit nuclear volume is of importance, and in the present experiments this density is approximately the same for both diploid and tetraploid cells as the 4n nuclei are roughly 2.2 times the volume of the 2n nuclei. In light of these considerations the observation that interchange frequency in 4n cells is only twice that in 2n cells "affords geometric evidence of the limitation of space over which chromosome breaks can interact to produce an exchange" (CONGER and JOHNSTON 1956).

The distribution of radiation-induced aberrations between cells is generally thought to be random and conform to a Poisson distribution (see discussion and review by LEA 1955). Recently, however, two important instances of deviation from Poisson distributions have been noted: JACKSON and BARBER (1958) found that the distribution of acentric fragments between root-tip cells of irradiated Allium seed showed a considerable degree of overdispersion in the data, and ATWOOD and WOLFF (1959) found that in Tradescantia microspores and in Vicia and Hordeum seed, the number of cells containing more than one chromosome interchange was always significantly smaller than that expected from a random distribution of aberrations, although other aberrations (isodiametric deletions) in the same cells did fit a Poisson distribution. In the present work the data from the colchicine experiment on the frequency-distribution of isochromatid and chromatid interchange aberrations is in many respects similar to that obtained by ATWOOD and WOLFF. The present results (Table 7) indicate that in both diploid and tetraploid cells the distribution of isochromatid aberrations is Poisson, whereas interchange aberrations do not fit a Poisson distribution, there being an excess of cells containing one interchange and a marked deficiency of cells containing more than one interchange. As ATWOOD and WOLF (1959) have pointed out, such a result indicates that interchanges are not induced randomly and that this is perhaps a consequence of the limitation in the number of sites in the nucleus where the chromatids of different chromosomes are sufficiently close together to undergo exchange. It is important to note that the distribution of interchanges in 2n and 4n cells is similar, as would be expected on the basis that chromosome density per unit volume is the same for both 2n and 4n nuclei.

SUMMARY

1. Radiation induced chromatid interchanges in root-tip cells of *Vicia faba* have been classified into eight, or ten, types on the basis of three criteria: (a) polarity of the chromosomes involved, (b) the manner in which the chromatid parts appear to have been exchanged, i.e. whether the exchange is of the U type or X type, and (c) whether the exchange is complete or incomplete.

2. Data from four experiments on interchanges in diploid roots indicated (a) that at least 80–90 percent of the chromosomes are polarized during interphase, and (b) that in about 70 percent of the interchanges observed at metaphase the exchange is of the U type. As a result of the high degree of chromosome polarity and high frequency of U type exchanges there is a significant excess of asymmetrical interchanges.

3. Although there were some slight indications of interaction between completeness and type of exchange, the results as a whole suggest that the three characters—polarity, type of exchange, and completeness—are independent of each other.

4. It is suggested that the presence of the U and X type interchanges at metaphase does not result from differences in the arrangement of the chromatids involved at the time of initiation of the exchange, but rather is an expression of two mechanically different types of exchange in interphase.

5. A study was made of the frequencies of chromatid interchanges and isochromatid aberrations in diploid and induced tetraploid cells of roots which were subjected to colchicine for 3½ hours, 21½ hours prior to irradiation.

6. The relative frequencies of the eight classes of interchanges were found to be similar for tetraploid and diploid cells. As 4n cells would have passed through two interphases without an intervening polarizing anaphase, the fact that chromosome polarity in 4n cells is as frequent as in 2n cells, together with the observation that there is a large increase in the frequency of interchange between homologous chromosomes in 4n cells, implies that little chromosome movement occurs during interphase and throughout 'c'-mitosis.

7. Observations on the relative frequencies with which the arms of the metacentric (M) chromosomes and the acrocentric (S) chromosomes undergo interchange, indicate that in both diploid and tetraploid cells the frequency of interchange per chromosome is not directly proportional to metaphase length of the chromosomes.

8. The frequencies of chromatid interchanges and isochromatid breaks in tetraploid cells was found to be twice the frequency observed in diploid cells, i.e. the frequency per chromosome is the same for 2n and 4n cells. The distribution of isochromatid breaks between cells, in both 2n and 4n cell types, fitted a Poisson distribution, but the distribution of interchanges was found to be non random.

ACKNOWLEDGMENT

I am indebted to MR. T. R. L. BIGGER for his able technical assistance with the colchicine experiment, and to MR. D. WESTGARTH and MR. T. F. HOBSON for statistical advice and computation.

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