Chromosome Studies on *Trillium kamtschaticum* PALL. and Its Allies. XXV. The Effects of Some Chemicals on the Frequency of X-ray-induced Chromosome Aberrations*

By

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With respect to the rejoining mechanism of the broken ends induced by X-ray irradiation, WOLFF ('59, '60) recently reported that the rejoining is prevented by the postirradiative treatments with protein synthesis inhibitors, thus concluding that protein synthesis is necessary for the occurrence of rejoining.

As to the distribution of breaks over chromosome arms, it was often indicated that heterochromatic regions are more breakable than euchromatic parts (KAUFMANN '46, MOUTSCHEN & GOVAERTS '53). However, in *Trillium* chromosomes, DARLINGTON & LA COUR ('45) demonstrated that heterochromatic regions are unbreakable to X-ray irradiation when charged with nucleic acids (Cf. SWANSON '58).

Along this sort of investigation, the present experiments were attempted by us with an aim to decide whether the frequency of X-ray-induced aberrations and their distribution in heterochromatic and euchromatic regions are modified or not by the administration of some chemicals, i. e. chloramphenicol (a protein synthesis inhibitor), mitomycin C (an inhibitor of nucleic acid synthesis) and acriflavine (an inhibitor of respiration in bacteria).

Materials and Methods

Materials used in this study are the ovular cells of *Trillium kamtschaticum* PALL. ($2n=10$). The concentration of each chemical was decided from the results of preliminary examinations as follows: 520 μg/ml acriflavine, 1000 μg/ml chloramphenicol, 2 μg/ml and 20 μg/ml mitomycin C respectively. Each solution was prepared with tap water. Nearly all of the materials are the vigorous plants having two flower-bearing stems. One of the stems was chilled for 4 days

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at about 0°C in order to determine the distribution of heterochromatin in each chromosome. The other one was used for the irradiation treatments. The cut stems were placed either in each active solution or in water, for 24 hours respectively, the latter being utilized as X-ray control. Six hours after the initiation of the chemical treatments the materials were irradiated with X-rays except some control plants in each set of chemical treatments. Radiation consisted of 180 kV X-rays filtrated through 0.5 mm Cu and 1.0 mm Al. The dosage of X-ray irradiation was 60 r. Each solution was replaced with water 18 hours after irradiation, that is, 24 hours after the beginning of chemical treatments. Ovular cells were fixed in La Cour 2BE forty two hours after irradiation, hydrolysed with 1N HCl and squashed after staining with leuco-basic fuchsin.

Results

1) *Abnormality caused by the simple chemical treatments (chemical control)*

The chemical treatments without irradiation caused a slight degree of injuries, such as slight contraction of chromosomes by chloramphenicol and slight stickiness of chromosomes and protuberance of nucleus at prophase or resting stages by mitomycin in a few cells (Table 1, Fig. 6). It is noteworthy

TABLE 1. Frequency of cells with chromosomal aberrations caused by mitomycin C, chloramphenicol and acriflavine (duration of treatment, 24 hrs: observations at 24 hrs after the end of the treatments).

Treatments	Metaphase				Ana- or Telophase			Total	% aberrant
	Normal	Stickiness	Contraction	Breakage	Normal	Stickiness	Bridge and fragment		
Mito. (20 μg/ml)	4	2	0	0	47	2	0	55	7.27
Mito. (2 μg/ml)	5	0	0	0	48	0	0	53	0.00
Chloram. (1000 μg/ml)	2	0	1	0	42	0	0	45	2.22
Acrif. (520 μg/ml)	7	4	0	0	52	3	0	66	10.60

Mito. = Mitomycin C: Chloram. = Chloramphenicol: Acrif. = Acriflavine.

that no breakage of chromosomes was observed in any of these treatments. WILSON & BOWEN ('51, '54) has reported that chloramphenicol has no ability to induce chromosome breakage. In the case of mitomycin C, however, KOBAYASHI ('59) reported its effect on animal sarcoma cells, showing the extreme fragmentation of metaphase chromosomes; on the contrary, in plant cells treated with 0.2, 2 and 20 μg/ml solutions for 8, 16 and 24 hours, we found neither fragment nor bridge even after 32 hours at anaphase and telophase.

Cytological effects of acriflavine or tripaflavine, was studied by BAUCH ('47) who showed that acriflavine causes the stickiness and clumping of chromosomes (Cf. BIESELE '58). When the roots of the present material *Trillium* were immersed for 3 hours in 2×10^{-3}, 2×10^{-4} and 2×10^{-5} mol solutions of acriflavine, mitotic configurations could not nearly observed in the preparations made at 18 hours after the end of treatment. Only rarely the metaphase cells showing extreme contraction of chromosomes were met with. On the other hand, in the ovular tissue preparations of the plants treated for 24 hours with 2×10^{-5} mol solution, through stem cut method, aberrant configurations were seldom observed. Accordingly, it may be regarded that chromosome breakage is not induced in so far as these concentrations adopted in the present experiments are concerned.

2) *Abnormality in X-ray treatment.*

Metaphase. Forty two hours after irradiation, chromatid recombination (Figs. 3, 5 & 7), chromatid-chromosome translocation, chromosomal translocation, chromatid breakage (CTB) and isochromatid breakage (CMB) (Figs. 4 & 5) were usually observed at metaphase. But the stickiness of chromosomes was hardly recognized. A dikinetic chromosome was met with only once in the combination treatment with 20 μg/ml mitomycin and X-rays (Fig. 3). The cells with these aberrations are likely to be the first division after irradiation, because the elimination of akinetics is not recognized, though chromosomal breaks are frequently observed. Sometimes, the association of kinetochores between two homologous chromosomes, and between two non-homologous ones were observed. Moreover, there were some configurations indicating the recombination of chromatids within the kinetochore region (Fig. 7). Some configurations showed the reunion between the broken ends of each arm of non-homologous chromosomes after the two independent breakages within kinetochore region.

In scoring the data on breakage, the combined treatment with acriflavine and X-rays was excluded and furthermore, as to the distribution of breaks, the case of chloramphenicol and X-rays was also excluded, because in these the frequency of aberrations scored was not enough to analyse. Since perfect cells with normal chromosome complements were omitted from scoring, it is not conclusive whether the frequency of breakages in metaphase chromosomes is modified or not by the mitomycin or chloramphenicol treatment.

Within the cells with aberrations, the distribution of types of breakage and rejoining, i. e. simple breaks (CTB, CMB) and rejoined ones (rB′, rB″), was studied in these treatments (Table 2). With regard to the isochromatid

TABLE 2. Frequency of simple chromatid and isochromatid breaks and chromatid and isochromatid breaks involved in recombinations at metaphase in various treatments. (60 r, 42 hours after irradiation)

Treatments	Total metaphase cells with aberrations	Simple chromatid breaks (CTB)	Rejoined chromatid breaks (rB′)	Simple iso-chromatid breaks (CMB)	Isochromatid breaks rejoined with chromatid breaks (rB″)	rB′/CTB	CTB/CMB
X-ray	44	44	4	35	0	0.091	1.26
X-ray & Mito. (20 μg/ml)	30	27	7	27	1	0.259	1.00
X-ray & Mito. (2 μg/ml)	56	61	11	55	3	0.180	1.11
X-ray & Chloram.	13	17		7	0	0.471	2.43

Mito.=Mitomycin C: Chloram.=Chloramphenicol.

breaks involved in interchange, only the breaks (rB″) rejoined with simple chromatid breaks (rB′) were recorded, because the identification of reciprocal translocations is somewhat difficult. Although the samples presented in this table are of small size, especially small in X-rays and chloramphenicol treatment, certain increase of two-hit aberrations was shown in the cases of the combined mitomycin or chloramphenicol treatment. The rB′/CTB ratio is increased from 0.091 to 0.259 or 0.180 by the mitomycin treatments, and it reaches to 0.471 by the application of chloramphenicol. The CTB/CMB ratio is also obtained in each set of the treatments. It is 1.26 in simple irradiation case, and 1.00 and 1.11 in the two X-rays-mitomycin treatments.

TABLE 3. Relative length of each chromosome in unchilled cells of *Trillium* (average of two homologous chromosomes).

Cell No.	Chromosomes				
	A	**B**	**C**	**D**	**E**
XM-I-1	27.90	18.27	17.88	22.40	13.56
XM-I-3	29.93	18.49	20.95	17.96	12.68
XM-I-3′	32.52	18.09	18.78	18.78	11.83
XM-I-4	29.75	19.00	19.00	16.82	15.42
XM-II-2	31.76	18.41	17.40	18.41	14.02
XM-II-2′	28.46	19.24	20.44	18.64	13.23
XW-1	31.22	19.60	17.42	18.51	13.25
XW-2	33.93	19.76	16.77	18.96	10.58
XC-1	31.31	17.85	19.19	16.84	14.81
XC-1′	30.86	19.75	17.28	17.28	14.81
Mean	30.76	18.85	18.51	18.46	13.42

Next, it was studied whether the breaks are distributed at random or specifically over the euchromatic and heterochromatic segments. According to DARLINGTON & LA COUR ('40, '41) and HAGA & KURABAYASHI ('53, '54), the heterochromatic segments in *Trillium* chromosomes reveal themselves as Feulgen negative parts under cold temperature (Fig. 2). Wilson & BOOTHROYD ('41), BAILY ('49) and SHAW ('59) recognized the less contraction of heterochromatic segments under such a condition. The present measurement of the relative chromosome length in the complement, however, did not show so conspicuous difference in contraction between chilled and unchilled cells (Tables 3 & 4), excepting that the relative length of the chilled **D**-chromosome, which is characterized by the longest heterochromatic region, was a little longer than

TABLE 4. Relative length of each chromosome in chilled cells of *Trillium* (average of two homologous chromosomes).

Cell No.	Chromosomes				
	A	B	C	D	E
XM-I-1	29.54	16.88	19.41	21.31	12.87
XM-I-2	26.36	21.64	16.73	20.73	14.55
XM-I-3	31.00	17.40	17.40	20.80	13.40
XM-I-11	30.08	22.61	16.86	19.54	10.92
XM-II-1	37.08	17.95	18.46	14.26	12.25
XM-II-2	28.28	18.97	18.45	22.07	12.24
XM-II-3	26.48	19.69	19.69	17.72	16.41
XM-II-12	32.56	19.77	16.94	18.11	12.62
XW-1	27.85	21.27	19.08	19.74	12.06
XW-2	28.80	17.55	17.36	20.32	15.98
XW-13	30.82	19.96	17.96	17.74	13.53
XC-1	30.05	19.34	18.83	20.38	11.40
Mean	29.91	19.42	18.10	19.39	13.19

TABLE 5. Relative length of euchromatic and heterochromatic regions of chilled chromosomes in each cell of *Trillium* (%).

Cell No.	Euchromatic region	Heterochromatic* region
XM-I-1	83.97	16.03
XM-I-2	83.64	16.36
XM-I-3	81.20	18.80
XM-II-1	83.05	16.95
XM-II-2	81.55	18.45
XM-II-3	80.31	19.69
XC-2	76.00	24.00
XW-1	80.26	19.74
XW-2	78.30	21.70
XW-13	80.90	19.10
XM-I-11	79.50	20.50
XM-II-12	76.70	23.30
Mean	80.4	19.6

* including kinetochore regions.

that of the untreated chromosome. For convenience' sake the kinetochore regions are included in the category of heterochromatin. The ratio of the length of total euchromatic segments to that of total heterochromatic ones within the complement was shown to be 80.4:19.6 from the measurements in 12 cells (Table 5).

The distribution of simple breaks over the heterochromatic and the euchromatic regions of chromosomes is shown in Table 6. The heterochromatic

TABLE 6. Frequency of X-ray-induced breaks in the euchromatic and heterochromatic regions in the completely analyzed cells.

Breaks	Treatments		Euchromatic	Euchromatic or heterochromatic	Heterochromatic (Including kinetchore)	Total breaks
Chromatid breaks (CTB)	X-ray		38 (86.4)*	2	4 (9.1)	44
		expect.**	33.8		8.2	
	X-ray & Mito.***		64 (72.7)	9	15 (17.1)	88
		expect.	63.5		15.5	
	X-ray & Mito. (20 μg/ml)		22 (81.5)	2	3 (11.1)	27
	X-ray & Mito. (2 μg/ml)		42 (68.9)	7	12 (19.7)	61
Chromosome breaks (CMB)	X-ray		22 (62.9)	4	9 (25.7)	35
		expect.	24.9		6.1	
	X-ray & Mito.		42 (51.2)	6	34 (41.5)	82
		expect.	61.1		14.9	
	X-ray & Mito. (20 μg/ml)		11 (40.7)	2	14 (51.9)	27
	X-ray & Mito. (2 μg/ml)		31 (56.4)	4	20 (36.4)	55

* Numbers within parenthesis representing break percentage.
** Representing expected number based on random distribution.
*** Mito. = Mitomycin C.

regions of *Trillium* chromosomes are generally located at the proximal parts to the kinetochore. However, there are small euchromatic regions between kinetochores and heterochromatic segments, and sometimes small spherules of euchromatin within heterochromatic regions. Accordingly, in the category of heterochromatic breaks shown in Table 6 such breaks as occurred in these euchromatic parts within the heterochromatic regions would have been included, although they would be negligibly small. As to chromatid breakage, it seems from Table 6 that the breaks are distributed at random. Chi-square tests were made for random distribution. And these values were 2.67 and 0.02 in each

of the simple irradiation and the combined mitomycin treatment, respectively. In the case of simple irradiation, the number of breaks located in euchromatic regions seems to be a little more than the expected values based on the random distribution, and less in heterochromatic parts, but the deviation from this distribution is not significant ($0.20>P>0.10$). On the contrary, in the case of isochromatid breakage it is shown that the breaks are more frequent in heterochromatic regions, when mitomycin treatments were combined with irradiation. In the mitomycin- X-ray treatment the deviation is highly significant ($P<0.01$), although in the simple irradiation it is not significant ($0.20>P>0.10$).

Anaphase and Telophase. Almost all of the abnormalities at anaphase or telophase were found to be structural changes of chromosome induced by irradiation as in the case at metaphase. In the simple irradiation treatment

TABLE 7. Frequency of aberrant cells with chromatid bridges in each treatment.

Treatments	1B*	2B	3B	4B	Total bridges	Total cells observed	Bridges per 100 cells
X-ray	248	47	2	0	348	1003	34.7
X-ray & Mito. (20 μg/ml)	332	51	12	1	474	1724	27.5
X-ray & Mito. (2 μg/ml)	190	21	6	0	250	1046	23.9
X-ray & Chloram. (1000 μg/ml)	97	17	5	0	146	478	30.5
X-ray & Acrif. (520 μg/ml)	170	36	2	0	248	1014	24.5

* B=Bridge(s).
Mito.=Mitomycin C: Chloram.=Chloramphenicol: Acrif.=Acriflavine.

the frequency of the cells having chromosome aberrations, if any, is 70.6 per cent at anaphase and telophase as averaged from two plants. When mitomycin was used in combination with irradiation, the frequency is 67.9 per cent (average from 5 plants) and 71.5 (from 3 plants) in the treatments with 20 and 2 μg/ml respectively. In the former, one of the five plants showed extremely low aberration frequency (39.9%). If this plant be excluded, the frequency would be 74.3 per cent. In the X-ray-chloramphenicol and X-ray-acriflavine treatments, these frequencies are 68.4 and 63.0 per cent, respectively. Thus, when chloramphenicol and acriflavine are combined with X-rays, there is a slight decrease in the aberration frequencies, although these frequencies seem not to be significantly different from each other. Table 7 represents the frequencies of chromatid bridges at anaphase and telophase in various treatments. From the data it will be recognized that the bridge formation decreases

by the combined chemical treatments. Especially, this is lower in frequency in the combined mitomycin treatments than in the simple irradiation, though the total frequencies of all types of chromosome aberrations increase slightly.

In order to obtain some informations on the nature of inhibition of sister reunion (SR) at broken ends, the following three types were picked up and recorded: the cells with only two isomorphic akinetic fragments (2Fi), those with one bridge and two isomorphic akinetic fragments (1B+2Fi), and those with one bridge and one akinetic fragment (1B+1F) (Table 8: Figs. 8—13).

TABLE 8. Frequency of anaphase and telophase cells with isomorphic fragment pair (2Fi), one bridge and a pair of isomorphic fragments (1B+2Fi), and one bridge and one fragment (1B+1F) in each set of treatments.

Treatments	2Fi	1B+2Fi	1B+1F	Total
X-ray	23 (13.0)*	20 (11.3)	134 (75.7)	177
X-ray & Mito. (20 μg/ml)	76 (30.3)	33 (13.1)	142 (56.6)	251
X-ray & Mito. (2 μg/ml)	59 (37.6)	26 (16.5)	72 (45.9)	157
X-ray & Chloram. (1000 μg/ml)	12 (18.2)	8 (12.1)	46 (69.7)	66
X-ray & Acrif. (520 μg/ml)	24 (16.8)	18 (12.6)	101 (70.6)	143

* Numbers within parenthesis representing break percentage.
Mito.=Mitomycin C: Chloram.=Chloramphenicol: Acrif.=Acriflavine.

The following three consequences after an isochromatid breakage, *SU** in which the SR takes place in both kinetic and akinetic fragments, *NUd** in which the SR takes place only in the kinetic fragment, and *NUpd** in which no SR takes place, will result in (1B+1F)-, (1B+2Fi)-and (2Fi)-type aberrations at anaphase, respectively. And *NUp**, the sister reunion only at the distal ends, will produce the ana- or telophase cells with only one akinetic fragment (1F) (Fig. 1).

On the other hand, (1F)-, (2Fi)-, (1B+1F)-or (1B+2Fi)-type will be also produced from certain proportion of chromatid-chromosome and chromosome-chromosome translocations. However, the data of metaphase observation show that these types of translocation are of infrequent occurrence. Similarly chromatid-chromatid translocation, which is shown to be relatively frequent at metaphase, is concerned with the yielding of these types of ana- or telophase aberrations. As to the chromatid-chromatid exchanges, however, there is a

* The nomenclatures adopted here, i.e. *SU*, *NUd*, *NUpd* and *NUp*, are those proposed by CATCHESIDE, LEA & THODAY ('46).

case in which neither bridge nor akinetic fragment is produced. Even in the case of dikinetic chromatid exchange, the bridge will not be produced when both kinetochores of the dikinetic chromatid involved have moved toward the same pole side. And if the ana- or telophase cells having two akinetic fragments within a cell result from the imperfect recombination of this chromatid translocation, the case of two isomorphic fragments, i.e. same length fragments, would be a matter of rare occurrence. Similarly only rarely two or more isomorphic fragments will originate from the independent chromatid breakages. Consequently it will be recognized that nearly all of the ana- or telophase cells of (2Fi)-, (1B+2Fi)- and (1B+1F)- types are the resultant aberrant cells of simple isochromatid breakage.

TABLE 9. Frequency of sister reunion at the broken ends in each set of treatment (inferred from the data presented in Table 8).

Treatments	Sister reunion (SR)	Non-sister reunion (NSR)	SR/NSR
X-ray	288 (81.4)*	66 (18.6)	4.38
X-ray & Mito. (20 μg/ml)	317 (63.1)	185 (36.8)	1.72
X-ray & Mito. (2 μg/ml)	170 (54.1)	144 (45.8)	1.18
X-ray & Chloram. (1000 μg/ml)	100 (75.8)	32 (24.3)	3.13
X-ray & Acrif. (520 μg/ml)	220 (76.9)	66 (23.1)	3.33

* Numbers within parenthesis representing relative ratios (%).

In the case of simple irradiation, the ratio, (2Fi) : (1B+2Fi) : (1B+1F), is 13.0 : 11.3 : 75.7. The ratio alters to 30.3 : 13.1 : 56.6 in the 20 μg/ml mitomycin and X-ray treatment, and 37.6 : 16.5 : 45.9 in the 2 μg/ml mitomycin and X-ray one. Moreover, the ratios in chloramphenicol- and acriflavine-X-ray treatments are 18.2 : 12.1 : 69.7 and 16.8 : 12.6 : 70.6, respectively.

Assuming that all of (2Fi), (1B+2Fi) and (1B+1F) aberrations observed result from the isochromatid breakage, according to the various consequences of SR, the extent of the failure of SR at the broken ends could be estimated. The ratio of the frequency of SR to that of its failure, i.e. SR/NSR, is 4.38 in the simple irradiation treatment, while in the combined irradiation treatments with the chemicals the ratio is 1.72 in 20 μg/ml, 1.18 in 2 μg/ml mitomycin, 3.13 in chloramphenicol, and 3.33 in acriflavine (Table 9). Thus there is certainly a general tendency toward the decrease of frequency of SR by the combined chemical treatments, especially in the mitomycin treatments.

The fact that the combined treatments considerably reduces the formation of chromatid bridge may be also explained as the results of the inhibition of

reunion by mitomycin.

Furthermore, if one assumes that the (1F)-type cells observed derived from only both cases of simple chromatid breakage and the isochromatid breakage followed by *NUp*, the proportion of the (1F) cells resulting from the latter case to the all (1F) cells scored can be roughly estimated from the ratio of simple isochromatid breaks to chromatid ones observed at metaphase (column 3 in Table 10). Including the frequencies of those cells of the (1F)-type resulting from an isochromatid breakage, the ratio SR/NSR could be also obtained

TABLE 10. Ratios of sister reunion to non-sister reunion at broken ends and of sister reunion at akinetic fragment (SRC_0) to that of kinetic fragment (SRC_1) (including presumed numbers of 1F-rt*).

Treatments	2Fi ($NSRC_0$) ($NSRC_1$)	1Frt* (SRC_0) ($NSRC_1$)	1B+2Fi (SRC_1) ($NSRC_0$)	1B+1F (SRC_0) (SRC_1)	Total	SR/NSR	SRC_0/SRC_1
X-ray	23	95 (215)**	20	134	272	2.38	1.49
X-ray & Mito. (20 μg/ml)	76	169 (337)	33	142	420	1.37	1.78
X-ray & Mito. (2 μg/ml)	59	139 (264)	26	72	296	1.09	2.01

* Indicating presumed number of reunion-type fragment calculated from the ratio of CMB to CTB at metaphase.

** Numbers in parenthesis representing total number of cells with one fragment at ana- and telophase.

Mito.=Mitomycin C.

(column 7 in Table 10). From these values it is shown again that the combined mitomycin treatments increase the extent of the failure of SR. In this case, an information is obtained as to which of akinetic and kinetic fragments are more favourable to SR. The SRC_0/SRC_1 ratios are 1.49, 1.78, 2.01 in the simple X-ray, in the combined irradiations with 20 μg/ml, and 2 μg/ml mitomycin, respectively. Accordingly, it is conceivable that SR takes place a little more frequently at the broken ends of akinetic fragments, as previously reported by various investigators.

Discussion

The present data from the observations on metaphase chromosomes indicate an increase in frequency of rejoined breaks by the combined mitomycin or chloramphenicol treatments, while those from anaphase or telophase chromosomes show a decrease in frequency of the SR at broken ends. It must be then questioned how both the results be combined into one. The most plau-

sible explanation will be given by assuming certain inhibition effect of mitomycin and chloramphenicol on the rejoining of broken ends; by those additional treatments their rapid rejoining is inhibited and they are kept open for longer period and their rejoinable period is also prolonged, thus leading to more frequent opportunity for them enough to interact with each other. In almost all the cells reaching to the stage of ana- or telophase, 42 hours after irradiation, the chromosomes may have undergone the anaphase separation before the recovery of the rejoinability of broken ends is accomplished.

It was proposed by THODAY ('52) that the SR arises from independent breakage of the two chromatids of the same chromosome after splitting. On the contrary, DARLINGTON & LA COUR ('45) and DARLINGTON & KOLLER ('48) hold the view that isochromatid breakage does not occur, and all isolocus breaks originate before the chromosome splitting, and the SR results from the impairment of chromosome reproduction by irradiation (Cf. THODAY loc. cit.). In the present materials, it seems likely that almost all of isolocus breaks were produced before the splitting of chromosomes. With the assumption that the impairment of reproduction results in the SR, however, the present data do not agree. Because, if one holds this assumption, one must interprete the fact of reduction of the SR by chloramphenicol and mitomycin C as due to the acceleration—not the inhibition (!)—of chromosome reproduction.

In the cells which have been attained to ana- or telophase at this time of fixation, the SR has been more frequently inhibited by the combined mitomycin treatments than by chloramphenicol ones. This fact shows that the inhibition effect of mitomycin on rejoining is somewhat stronger than that of chloramphenicol. And this may also account for the fact that in the metaphase cells elapsed 42 hours after irradiation the frequency of breaks involved in the formation of interchange is higher in the X-ray-chloramphenicol treatments than in the X-ray-mitomycin ones.

Thus the present data will be well explained by the effect inhibiting rapid rejoining of these metabolic inhibitors, as proposed by WOLFF ('59, '60) and further by the prolongation of both periods, open and joinable. Furthermore it is apparent from the results of the combination treatment with chloramphenicol that protein synthesis is necessary for rejoining to occur. According to Wolff, RNA and DNA syntheses do not seem to be necessary for the rejoining, although protein synthesis seems to be involved. He deduces this conclusion from the fact that the incorporation of C^{14} labeled glycine into the protein is prevented when the soaked *Vicia* seeds are treated with 300 μg/ml chloramphenicol and this chemical at 300 μg/ml does not inhibit the incorporation of P^{32} into the nucleic acids, and also that tritiated thymidine is not incor-

porated into cell nuclei at such particular time as in the experiments by Wolff and his collaborators (Cf. WOLFF '60).

Mitomycin C, an antibiotic substance extracted from *Streptomyces caespitosus*, is known to inhibit specifically the DNA synthesis of *Escherichia coli* at certain concentration (SHIBA, TERAWAKI, TAGUCHI & KAWAMATA '58, SEKIGUCHI & TAKAGI '60). If in higher plants the same effect of mitomycin C as in bacteria is assumed, the possibility that DNA synthesis is involved in the rejoining of broken ends can not be excluded.

BEATTY & BEATTY ('59) reports that respiration process is also necessary for the rejoining. The present data indicate that a respiratory inhibitor in bacteria, acriflavine, decreases the frequency of the SR, though only slightly, although it is not known as yet whether acriflavine is effective in inhibiting respiration of the present material. According to BETZ ('53), however, acriflavine combines with nucleic acid (Cf. BIESELE '58). If this is operative in the present experiment, a decrease in frequency of the SR by acriflavine would also suggest the involvement of nucleic acid in the rejoining of induced breaks.

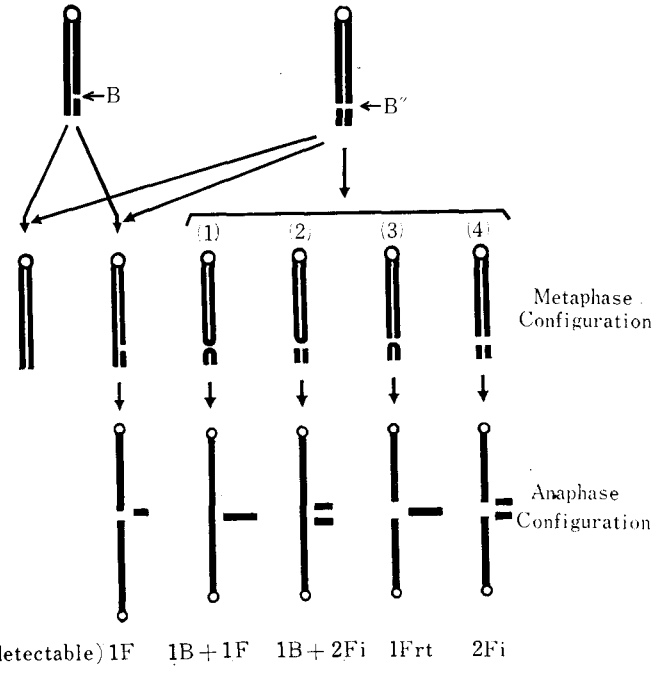

Fig. 1. Diagrammatic illustration of the consequences of isochromatid and chromatid breakage (after effective splitting).
B′ = Chromatid Breakage: B″ = Isochromatid Breakage

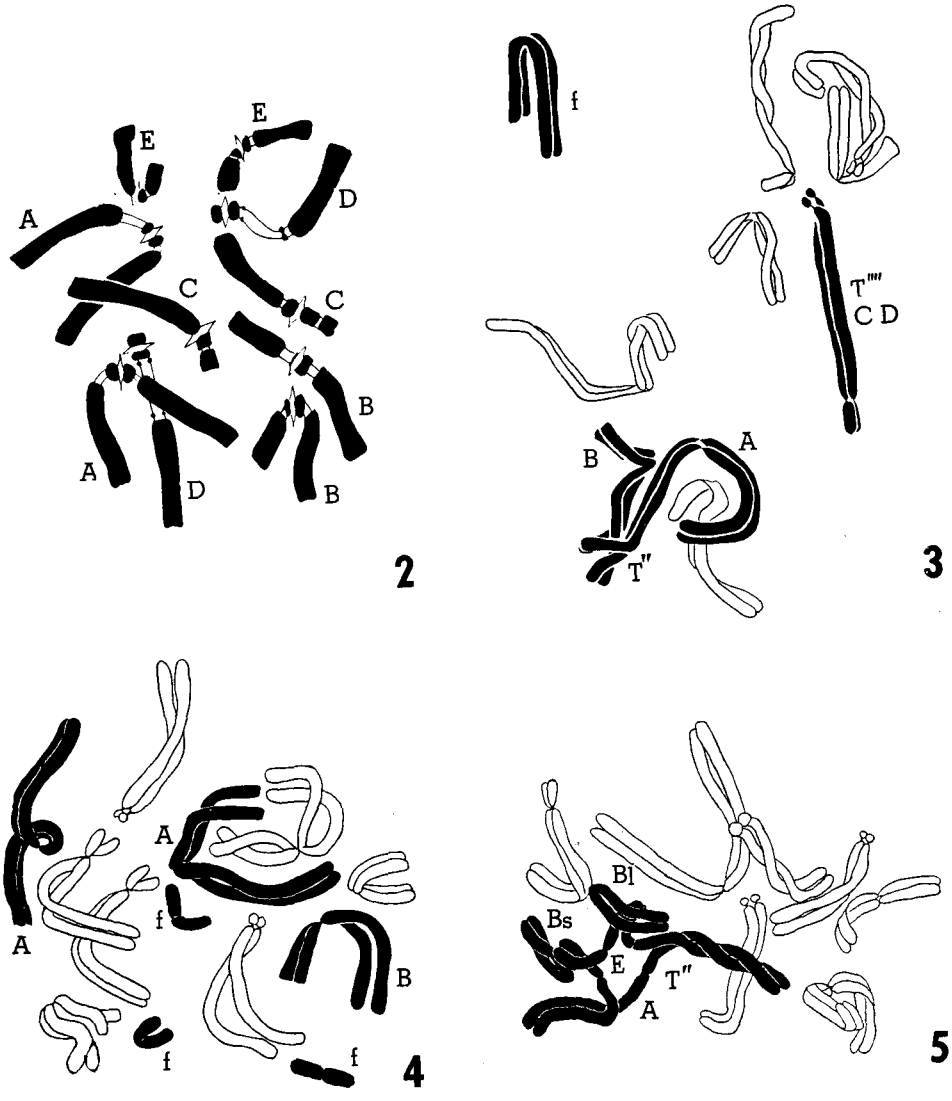

Fig. 2, chromosomes in a chilled plant, illustrating the heterochromatic regions. **3,** a dikinetic chromosome (**C**- and **D**-chromosomes) and an akinetic fragment (f), and chromatid-chromatid translocation (**A**- and **B**-chromosomes). **4,** three isochromatid breakages (**A**-, **A**- and **B**-chromosomes). **5,** an isochromatid breakage (**B**-chromosome) and chromatid-chromatid translocation (**A**- and **E**-chromosomes).

Next, how is the combined mitomycin treatment responsible for the increase in the relative frequency of simple breaks in heterochromatic region? A considerable number of potential breaks produced by X-rays may restitute and may not be realized as visible aberrations. Is in heterochromatic regions rejoining or restitution of broken ends remarkably inhibited by mitomycin? In the scored metaphase cells having chromosome aberrations, the frequencies of breaks per unit length in heterochromatic and euchromatic regions were calculated and compared with each other, and from this it was found that in both cases of chromatid and chromosome breakages (B′, B″) the frequency in the heterochromatic regions is approximately doubled by the application of mitomycin, whereas that in the euchromatic regions is kept nearly unaltered. This may be ascribed to the more effective inhibition of mitomycin to rejoining in heterochromatic regions. Since, however, in the present study, the accurate frequency of breaks in heterochromatin and euchromatin was not scored, and the number of the breaks detected was not so sufficient as to allow statistical analysis, any decisive conclusion must be waited until a more detailed study is accomplished.

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SUMMARY

1) The modifying effects of mitomycin C, chloramphenicol and acriflavine on the frequency of X-ray-induced chromosome aberrations were investigated with the ovular tissue cells of *Trillium kamtschaticum* Pall. (2n=10).

2) Cytological observations were carried out on the metaphase and anaphase or telophase cells elapsed 42 hours after irradiation.

3) The results from the metaphase cells showed an increase of two-hit aberration yield by the chloramphenicol and mitomycin treatments (Table 2).

4) The distribution of simple chromatid or isochromatid breaks over the heterochromatic and the euchromatic regions of chromosomes were examined at metaphase. In so far as simple isochromatid breaks are concerned, the relative frequency of the breaks in heterochromatic regions was increased by the mitomycin treatments (Table 6).

5) From the data of the ana- or telophase cells, it is inferred that mitomycin, chloramphenicol and acriflavine treatments tend to inhibit the occurrence of sister reunion (Tables 7, 8, 9, & 10: Fig. 1).

6) It is supposed that sister reunion takes place a little more frequently in akinetic fregments than in kinetic ones (Table 10).

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Explanation of Plate

Plate I.

Fig. 6, protuberance of nucleus at resting stage caused by mitomycin treatment.

Fig. 7, recombination of chromatid within kinetochore region.

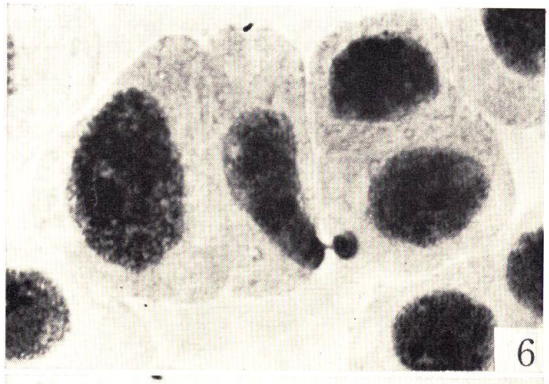
Fig. 8, telophase cell with an akinetic fragment (1F).

Fig. 9, telophase cell with a bridge and a fragment (1B+1F).

Figs. 10, & 11, telophase cell with a bridge and a pair of isomorphic fragments (1B+2Fi).

Fig. 12, telophase cell with two isomorphic fragments (2Fi).

Fig. 13, telophase cell with two isomorphic fragments (2Fi) and an akinetic fragment.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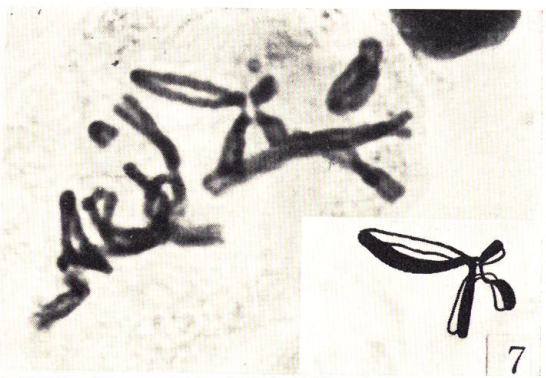
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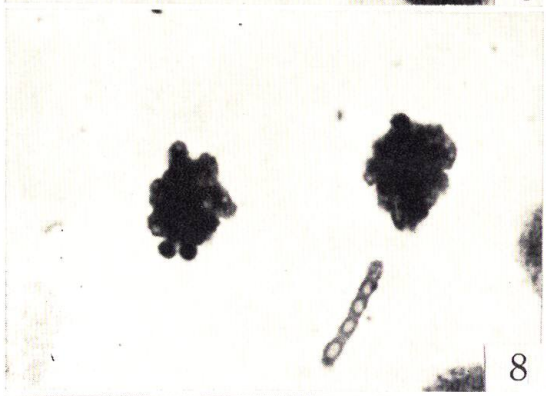
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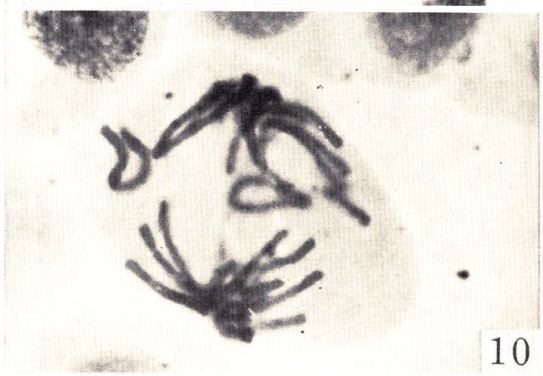
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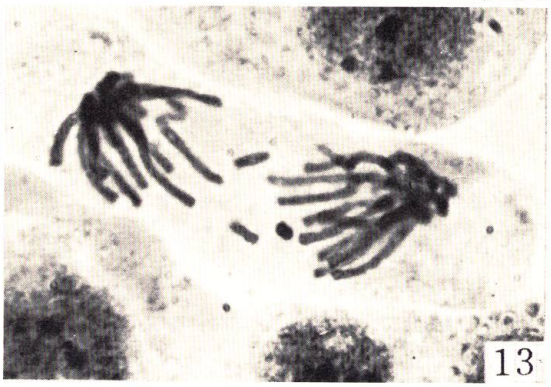
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