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Chromosome Studies on *Trillium kamtschaticum* PALL. and Its Allies. XXVIII. Modifying Effects of Chloramphenicol on X-ray-Induced Chromosome Aberrations*

By

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Although a number of contributions have hitherto been made on breakage and reunion of chromosomes following ionizing radiations, one of the most important problems which remains yet unsolved seems to be the chemical process of the rejoining or repair of broken ends. Fortunately, however, remarkable approaches have recently been made through some excellent procedures, such as the administration of various metabolic inhibitors and the fractionation of radiation doses. First, it was reported by WOLFF & LUIPPOLD ('55) and BEATTY & BEATTY ('59, '60) that the supply of energy is necessary for the process of rejoining. Moreover, WOLFF ('59, '60) concluded, from the experiments employing mainly chloramphenicol which is known to be a specific inhibitor of protein synthesis, that protein synthesis is necessary for the rejoining.

In the present experiments, the modifying effects of chloramphenicol on X-ray-induced chromosome aberrations were investigated with meiotic chromosomes of *Trillium*. As a tentative experiment, the effect of acriflavine, which is known to be a respiratory inhibitor in bacteria, was also studied.

Materials and Methods

Pollen mother cells of *Trillium kamtschaticum* PALL. ($2n=10$, 5II) were used throughout the experiments. The five chromosomes of the gametic set of the material are represented as **A**, **B**, **C**, **D** and **E** (Fig. 2). About ten days before the first metaphase of meiosis the plants were transferred from the field into the greenhouse (3/Dec., 1960). Four days later the cut stems bearing flower buds were inserted each for 10 hours in the solutions of chloramphenicol

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and acriflavine, and also in tap water, the last being employed as the X-ray control. Concentrations of chloramphenicol employed are 100, 500 and 1000 μg/ml, and that of acriflavine is 2×10^{-4} Mol. At the beginning of the treatments the PMCs of the plants were at the stage of prophase, as shown in Fig. 1. Seven hours after the treatment the materials were irradiated with X-rays except some plants which were used as the chemical control. For X-raying a dose of 20 r was given at 180 kV, 6 mA, and at 8.1 r.p.m. dose rate, with a filtration of 0.5 mm Cu $\times$1.0 mm Al (half value layer, 0.95 mm Cu). The preparations were made as acetocarmine smears, and chromosome aberrations were scored at the 1st metaphase. In order to get fine structure of configurations the MATSUURA's pretreatment method with KCl aq. solution (MATSUURA 1944) and its modified method were applied just prior to fixation and staining with acetocarmine.

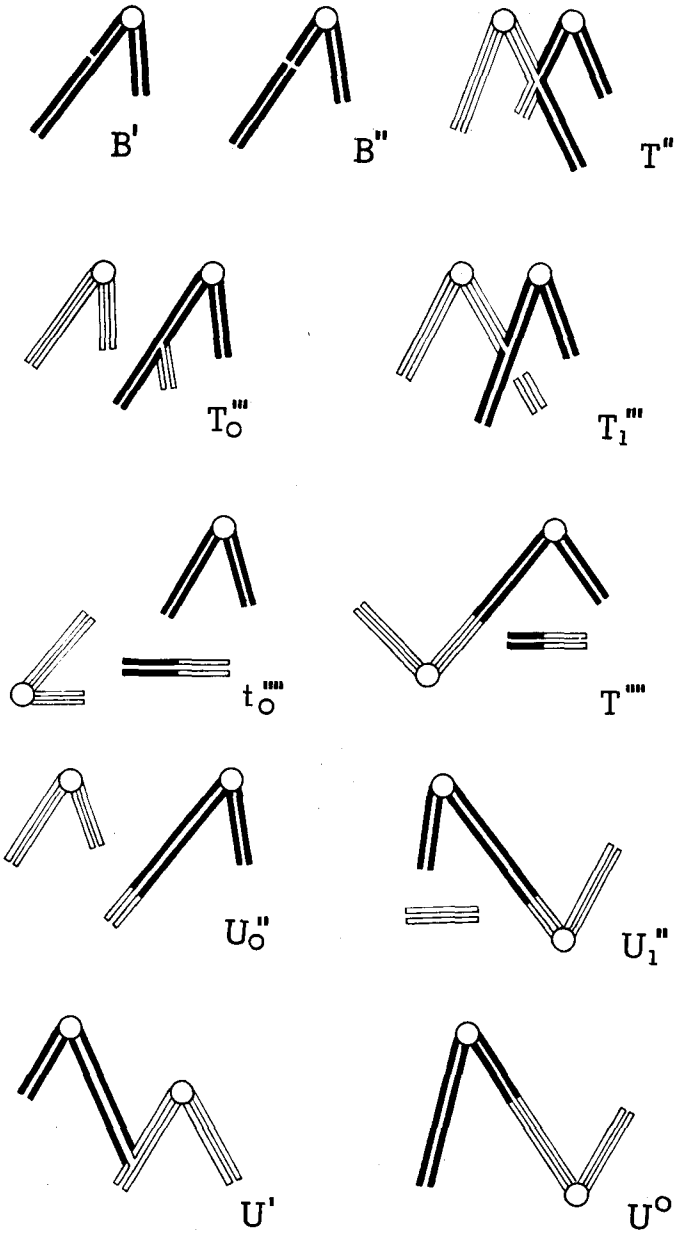
Observations and Discussion

1) Types of aberrant configurations induced by X-rays.

The types of chromosome aberrations induced by X-rays are dependent upon the division stages at which the cells were irradiated. For example, in the materials irradiated at diplotene, diakinesis and 1st metaphase, many configurations of half-chromatid recombination were observed (WILSON, SPARROW & POND '59, WILSON & SPARROW '60, MATSUURA, TANIFUJI, IWABUCHI & KANAZAWA in press). SAX ('38) demonstrated that the irradiation before the effective splitting of chromosomes gives rise to only iso-chromatid breaks, while the irradiation after the splitting yields only simple chromatid ones. In the present materials, however, there is no clear-cut shifting of chromosomal aberrations to chromatid ones. Even in the materials irradiated one or two months before the first division both types of chromatid and iso-chromatid aberrations were recognized (MATSUURA & HAGA '50, KURABAYASHI '58). This is true in the present materials irradiated about one week before 1st metaphase.

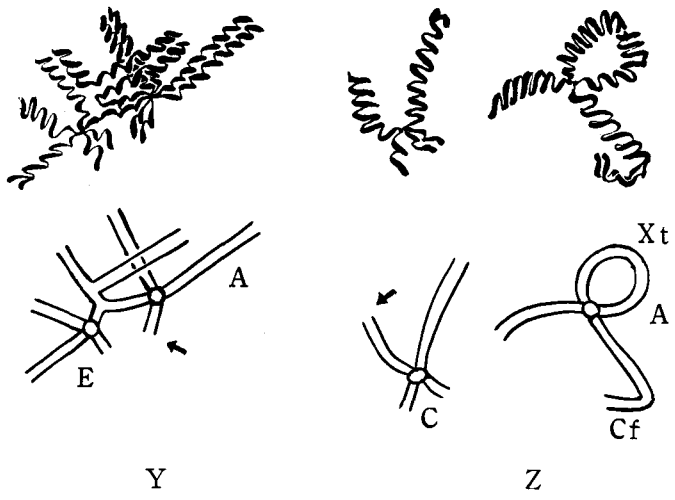
The chromosome aberrations which are detectable at 1st metaphase, in the both cases of simple X-ray treatment and the combined irradiation treatment with chemicals, were (i) simple iso-chromatid breaks (B″), (ii) simple chromatid breaks (B′) which are only rarely found probably due to the difficulty of the detection, (iii) chromatid-chromatid inter- (or intra-) changes (T″), (iv) chromosome-chromatid interchanges (T_0'', T_1''), and so on (Figs. 3, 4, 5, 6 & Text-fig. Y). The various types of the aberrations are diagrammatically illustrated in Text Figure X, and symbols are given respectively to them.

It must be noted here that the configurations showing union between broken ends and normal distal ends as reported by MATSUURA and HAGA ('50), were often observed (U′, U_1'' & U_0'' in Text-fig. X: Figs. 7, 8 & Text-fig. Z). Furthermore, unions between normal ends of non-homologous chromosomes



Text-figure X.
(Explanation in text)

(U^0) were sometimes met with, which are similar to that reported as self-union by DARLINGTON and LA COUR ('53) in the PMCs of *Tradescantia* and *Secale* after X-ray irradiation (Figs. 9 & 10). Cautious observations are required to identify the unions between two normal ends and those between broken ends and normal ones. If one or both of the chromatid breaks in two involved chromosomes occurred in very distal regions, the configuration which is origi-



Text-figs. Y & Z.

Text-figs. Y & Z. Semidiagrammatic representation of chromosome aberrations induced by X-rays. Fig. Y, U′ between short arm of **E**- and long arm of **A**-chromosome, and intrabivalent chromatid-chromosome interchange (T_6'') in **A**-bivalent. Fig. Z, union between an unbroken end of **A**-chromosome and an end (presumably broken end) of akinetic fragment (**Cf**) originated from an iso-chromatid breakage in a long arm of **C**-chromosome.

nally quadriradial (T″) might be sometimes misjudged as U′ or U^0 type. However, even the free part or parts distal from the broken points with length of only about half coil were often recognizable (Fig. 5). Accordingly, it may be true that sometimes the normal ends of chromosomes participate in the recombination events.

The frequency of these aberrant types except simple chromatid breakage (B′) is represented in Tables 1 and 2. The class of *other interchange aberrations* in Table 2 includes the configurations showing the indistinct type of interchange. In Table 1, all types of the interchanges are grouped in one

TABLE 1. Frequency of simple breakage (B″-s) and reunion (two-hit aberrations) at the 1st metaphase in PMCs of *Trillium kamtschaticum* after X-ray irradiation (20 r) and combined chemical treatments.

Treatment	Plant No.	Days after irradiation	Simple iso-chromatid breaks per 100 cells	Interchange aberrations per 100 cells	Total MI cells observed
20 r	Cox-1	6	11.77	6.72	119
	Cox-2	6	12.28	3.51	57
	Cox-4	6	12.62	8.60	523
	3 plants		12.22±0.20	6.28±1.21	699
Chloram. (100 μg/ml) & 20 r	Xc-1-1	6	10.29	9.31	204
	Xc-1-2	6	7.14	5.10	98
	Xc-1-3	8	10.13	11.81	237
	3 plants		9.19±0.84	8.74±1.60	539
Chloram. (500 μg/ml) & 20 r	Xc-2-2	5	10.71	10.95	411
Chloram. (1000 μg/ml) & 20 r	Xc-3-1	5	12.46	16.37	281
	Xc-3-2	5	8.99	9.36	267
	Xc-3-3	5	6.84	9.13	263
	Xc-3-4	6	6.52	16.96	230
	4 plants		8.70±1.18	12.96±1.86	1041
Acrif. (2×10^{-4}M) & 20 r	Xa-3-1	6	12.41	6.90	145
	Xa-3-3	8	15.42	8.88	214
	2 plants		13.92	7.89	359

Chloram. = chloramphenicol: Acrif. = acriflavine.

class as *interchange aberrations.*

2) Modifying effect of chloramphenicol on the frequencies of induced aberrations.

In the simple chemical treatments without irradiation, only a few breakages could be found. Only one chromatid break among total 332 MI cells, and only three akinetic fragments among 100 AI cells were met with in the 1000 μg/ml, and 500 μg/ml chloramphenicol treatments respectively. In the acriflavine treatment (2×10^{-4} Mol) only one iso-chromatid breakage was observed among 160 MI cells.

TABLE 2. Frequency of various types of interchanges scored at MI in PMCs of *Trillium kamtschaticum* after X-ray irradiation (20 r) and combined chemical treatments.

Treatments	Plant No.	Interchanges											Total
		T″ (B′–B′)	U′ (B′–Ne)	T_0''' (B′–B″)	T_1''' (B′–B″)	U_1'' (B″–Ne)	U_0'' (B″–Ne)	T‴‴ (B″–B″)	t_0'''' (B″–B″)	Tr (B″–B″)	U⁰ (Ne–Ne)	Other interchange aberrations	
20 r	CoX-1	4	2	—	—	—	—	—	—	—	1	1	8
	CoX-2	1	—	—	—	—	—	—	1	—	1	—	3
	CoX-4	20	12	—	1	—	1	—	—	—	2	9	45
		25	14	—	1	—	1	—	1	—	4	10	56
Chl. (100 μg/ml) & 20 r	Xc-1-1	13	3	—	—	—	—	—	—	—	2	1	19
	Xc-1-2	2	1	—	—	—	—	—	—	1	—	1	5
	Xc-1-3	16	4	1	—	—	1	—	—	—	—	6	28
		31	8	1	—	—	1	—	—	1	2	8	52
Chl. (500 μg/ml) & 20 r	Xc-2-2	22	11	2	2	—	1	—	—	—	—	7	45
Chl. (1000 μg/ml) & 20 r	Xc-3-1	15	7	4	—	1	1	4	4	—	2	9	47
	Xc-3-2	17	3	1	—	1	—	—	—	—	—	3	25
	Xc-3-3	19	4	—	—	—	—	—	—	—	1	—	24
	Xc-3-4	17	10	—	1	—	—	—	—	1	4	6	39
		68	24	5	1	2	1	4	4	1	7	18	135
Acrif. (2×10^{-4} M) & 20 r	Xa-3-1	7	2	—	—	—	—	—	—	—	—	1	10
	Xa-3-3	7	1	2	—	—	—	1	—	—	4	4	19
		14	3	2	—	—	—	1	—	—	4	5	29

Symbols in the 2nd column represent various types of interchanges shown in Text Fig. X.
B′ = chromatid broken end: B″ = iso-chromatid broken end: Ne = normal end of chromosome.
Chl. = chloramphenicol: Acrif. = acriflavine.

In the simple irradiation experiments, the frequency of simple iso-chromatid breaks is 12.22 per 100 cells, and that of interchanges, almost all of which are of true two-hit aberrations, is 6.28. However, the administering of chloramphenicol allows for the yields of interchange aberrations to increase, that is, in the combination treatments with 100, 500 and 1000 μg/ml chloramphenicol the frequency increased to 8.74, 10.95 and 12.96, respectively. On the contrary, the frequency of simple iso-chromatid breaks is decreased by the chloramphenicol treatment (Table 1).

In the experiments with acriflavine any modifying effect on the frequency of aberrations was not detected, at least so far as the concentration of 2×10^{-4} Mol is concerned.

An attempt was made in order to investigate whether or not the application of chloramphenicol modifies the frequency of the unions between two normal distal ends and between normal and broken ends. However, any positive evidence was not obtained (Table 3). Normal distal ends of chromosomes unite more frequently with the induced broken ends of chromatids than do so with normal ends of another chromosome, consequently triradial configurations being formed more frequently.

Frequencies of chromatid breaks and iso-chromatid breaks, both of which participate in the formation of true two-hit aberrations, were recorded in each treatment respectively (Table 4). Sometimes there were the configurations of two-hit aberrations in which some of the broken ends remained unrejoined (Fig. 8). In scoring the data, however, these configurations were not distinguished from their respective complete forms. In Table 4 are represented the number of rejoined chromatid breaks and of the broken ends originated from iso-chromatid breakage involved in reunion, and doubled number of simple iso-chromatid breakage. These numbers were compared with one another on the following reason. Two pairs of broken ends of chromatids which originated from an iso-chromatid breakage (B″) are both able to be involved in the formation of two-hit aberration. On the contrary, two broken ends of a chromatid arised from single chromatid breakage (B′) are possible to rejoin as one unit with another pair of broken ends of a chromatid.

From the data in Table 4, it is shown that a decrease in frequency of simple chromosomal broken ends and an increase of rejoined chromatid breaks by administering of chloramphenicol are remarkable, but the frequency of iso-chromatid broken ends involved in the formation of two-hit aberration is slightly raised. With respect to the increase of rejoined chromatid breaks, it is considered that simple chromatid breaks which were obscure before the parallelization of the spiral structure of chromatids become detectable by the formation

TABLE 3. Frequency of reunions between two normal ends of chromosomes and between broken ends and normal ones scored at MI in PMCs of *Trillium kamtschaticum* after X-ray irradiation and combined chemical treatments (per 100 cells).

Treatment	Number of plants observed	Total MI cells observed	Ne-Ne (U^0)	Ne-B′ (U')	Ne-B″ (U''_0, U''_1)	Total
20 r	3	699	0.57	2.00	0.14	2.71
Chloram. (100 μg/ml) & 20 r	3	539	0.37	1.48	0.19	2.04
Chloram. (500 μg/ml) & 20 r	1	411	0.00	2.68	0.24	2.92
Chloram. (1000 μg/ml) & 20 r	4	1041	0.67	2.31	0.29	3.27
Acrif. (2×10^{-4} M) & 20 r	2	356	1.11	0.84	0.00	1.95

Chloram. = chloramphenicol: Acrif. = acriflavine. B′ = chromatid broken end: B″ = iso-chromatid broken end: Ne = normal end of chromosome.
Symbols within parenthesis represent various types of interchanges shown in Text-fig. X.

TABLE 4. Frequency of simple iso-chromatid breaks, rejoined chromatid breaks and rejoined chromosomal break ends at MI of *Trillium kamtschaticum* after various treatments. (per 100 cells)

Treatments	Number of plants observed	Total MI cells observed	Double number of simple iso-chromatid breaks	Number of simple chromatid breaks involved in interchanges	Number of chromosomal break ends involved in interchanges
20 r	3	699	24.89	7.30	0.38
Chloram. (100 μg/ml) & 20 r	3	539	19.30	11.69	0.93
Chloram. (500 μg/ml) & 20 r	1	411	21.41	11.68	0.97
Chloram. (1000 μg/ml) & 20 r	4	1041	17.68	13.64	3.27
Acrif. (2×10^{-4} Mol) & 20 r	2	359	28.41	8.36	1.67

Chloram. = chloramphenicol: Acrif. = acriflavine.

of chromatid interchange. The decrease in the frequency of unrejoined iso-chromatid broken ends might be in part due to the formation of two-hit aberrations with another iso-chromatid broken ends or with simple chromatid breaks. If one assumes the accelerating effect of restitution by this chemical, then it is expected that by the combined treatments almost all of the simple iso-chromatid broken ends are converted to the state of simple chromatid breakage (B′) or quite normal state by the restitution of one or two chromatids. On the other hand, a plausible explanation will be given by assuming the

conversion of some simple iso-chromatid breaks into the rejoined breaks involved in chromatid-chromatid interchange (T″). As WOLFF ('59, '60) has shown from the results of the radiation experiments using the somatic chromosomes of *Vicia*, the rapid rejoining of broken ends will be prevented and breaks will stay open for a longer period by chloramphenicol. After the open period the rejoinability will recover and further the joinable period following the recovery will also prolonged when chloramphenicol is administered, thus the chance of interaction between broken ends increases. Furthermore in the case of irradiation with chloramphenicol, even between two iso-chromatid breaks, i. e. between two non-sister chromatids of the two involved chromosomes, quadriradial (T″) formation will take place more frequently as well as between two chromatid breaks (B′). Later or simultaneously the rest breaks of chromatids which are not involved in the recombination will restitute. The perfect failure of rejoining seems to be infrequent in the present materials because the appearance of two akinetic fragments at 1st anaphase was rather infrequent and in almost all of cases akinetic fragments which are accompanied with one bridge were one in number. Furthermore, at 1st metaphase the configurations showing sister reunion were frequently observed.

By supposing the involvement of iso-chromatid breaks in the chromatid-chromatid interchanges the frequent appearance of T″ type aberrations becomes well understandable. But, if the akinetic fragments originated from iso-chromatid breakage have been released from the original position after a short period from the hits, the opportunity of the involvement in T″ formation will be lost. When, however, one considers the existence of potential breakage, although one does not well known about it, it seems likely that the release of the akinetics from the original position is infrequent in the case of the present experiment. And there is no evidence that such chromosome substance as the matrix of metaphase or anaphase chromosomes does not exist at prophase. Actually metaphase configurations in which akinetic fragments remained at the original position are frequently observed. Sometimes sister reunions are clearly illustrated in them (Cf. Fig. 5 in MATSUURA & HAGA '50).

Finally the number of hits within a cell must be considered. As shown in Tables 1 and 2, the frequency of detected breaks within a cell is of small order, from about 0.18 to 0.46 per cell. According to LEA ('55), only one-tenth of breaks are realized as visibly detectable aberrations, and the rest of them undergoes restitution. In the present materials undetectable breaks of chromatid (B′) which failed to rejoin with other broken ends would have been also numerous. Therefore, there would have been the breaks of the moderate number for the chance of interaction within a cell.

TABLE 5. Distribution of induced breaks in each chromosome of *Trillium*.

Type of aberrations	Treatments	Chromosomes				
		A	**B**	**C**	**D**	**E**
Simple iso-chromatid breaks	20 r	23	10	14	14	4
	20 r & Ch. 100 μg/ml	8	6	9	8	3
	20 r & Ch. 500 μg/ml	14	6	11	5	1
	20 r & Ch. 1000 μg/ml	30	13	9	17	4
	Total	75 (35.89)*	35 (16.75)	43 (20.57)	44 (21.05)	12 (5.74)
Rejoined chromatid breaks	20 r	16	13	7	22	7
	20 r & Ch. 100 μg/ml	23	9	13	20	6
	20 r & Ch. 500 μg/ml	16	13	18	15	2
	20 r & Ch. 1000 μg/ml	52	27	28	37	18
	Total	107 (29.56)	62 (17.13)	66 (18.23)	94 (25.97)	33 (9.12)
Rejoined iso-chromatid breaks	20 r	1	0	3	2	0
	20 r & Ch. 100 μg/ml	2	0	2	0	0
	20 r & Ch. 500 μg/ml	1	2	1	1	0
	20 r & Ch. 1000 μg/ml	8	7	1	5	4
	Total	12	9	7	8	4
Grand total		194 (31.75)	106 (17.35)	116 (18.99)	146 (23.90)	49 (8.02)
Relative length of chromosomes based on the gyre number (average from 18 cells)		30.13	19.10	19.10	18.47	13.20

* Numbers within parenthesis represent percentage.
Ch. = chloramphenicol.

3). Distribution of breaks in each chromosome.

Generally speaking, it have been shown that the distribution of breaks induced by X-rays is at random. In the meiotic chromosomes of *Trillium*, MATSUURA and HAGA ('50) illustrated from the data of bridges and loops at 1st anaphase the proportionality between the frequency of the aberrations and the length of chromosomes. Later KURABAYASHI ('58) showed that in the case of simple iso-chromatid breakage (B″) **B** and **E** chromosomes seem to be less breakable, although the frequency of those involved in recombinations (T″, T_1''', T_0''' & T‴″) is proportional to the length of chromosomes.

One of the initial aims of the present experiments was to test whether the distribution of breaks is modified or not by the combined chemical treatments. However, the available data are not sufficient to warrant any conclusion on this problem.

In scoring the data the breaks rejoined with normal distal ends are also included and both of chromatid and iso-chromatid ones are counted as one hit event. The distribution of induced breaks in each of the five chromosomes of

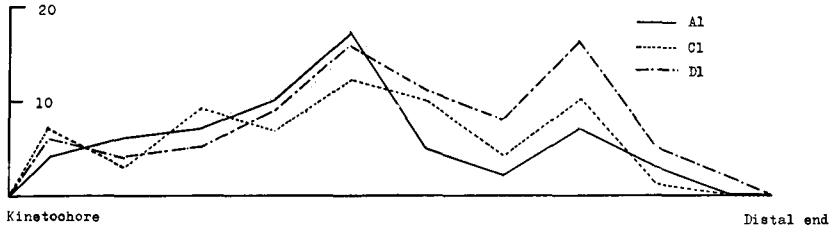
TABLE 6. Data on distribution of induced breaks along chromosome arms of *Trillium*. I) Simple chromatid breaks (B′)

Treatments	Chromosome arms							
	A	**Bl**	**Bs**	**Cl**	**Cs**	**Dl**	**El**	**Es**
X-rays	2/8	4/6				8/10		
X-rays & Chl. (100 μg/ml)						5/10		
X-rays & Chl. (500 μg/ml)	5/9 2/10			5/8		7/10		
X-rays & Chl. (1000 μg/ml)	4/8 3/7 5/9.5					4/9 0/?		

Chl. = chloramphenicol.

Each fractional number = $\frac{\text{No. of gyres between kinetochore and break point}}{\text{Total no. of gyres of whole arm}}$

the haploid set of *Trillium kamtschaticum* is shown in Table 5. With respect to simple iso-chromatid breakage (B″), the frequency of the breaks seems to be less in **B** and **E** chromosomes in the both cases of the simple irradiation and the combination treatments. The position of broken points on each chromosome arm is decided by the ratio of the number of gyres between the broken point and the kinetochore to the total number of gyres in the whole arm (Tables 6, 7, 8 & 9). Summing up all of breaks detectable, the frequency polygons of broken points along the arms of chromosomes are shown in Graph 1, only in the cases of the arm of **A** (sum of both arms), long arms of **C** and **D** chromosomes. As shown in the Graph, they seem to be nearly even distribution curves.



Graph 1. Frequency polygons of induced break points in chromosome arms. (Simple and rejoined broken ends of chromatids and iso-chromatids)

TABLE 7. Data on distribution of induced breaks along chromosome arms of *Trillium*. II) Rejoined chromatid breaks (rB′).

Treatments	Chromosome arms							
	A	**Bl**	**Bs**	**Cl**	**Cs**	**Dl**	**El**	**Es**
X-rays	4/8 2/6 3/7 5/8 3/7 1/7 6.5/10 6/8 4/8	2/6 3/6 4/5 4/6	2/5 0/4 2/3 2.5/3	2/8 4/8 5/8 2/10		4/9 6/10 4/9 2/7 6/10 6/8 5/7 8/11 0/8	2/4.5 2/5	
X-rays & Chl. (100 μg/ml)	1/8, 3/7 4/8, 0/10 6/8, 6/10 1/7 5/8 3/8 3/8 4/7 3/6 7/9	4/6 5/7		5/7 0/7 4/9 3/8	1/2	5/11 7/11 9/11 7/9 5/11 1/11 4/9 5/8 5/10 8/11		
X-rays & Chl. (500 μg/ml)	6/9 5/7 0/? 6/8 4/8 5/9 1/8 5/8	2/7 4/6		4/7 2/9 4/7 8/10 5/9		6/10 3/10 8/11 6/10 3/9 1/9 2/10 2/10 3/10 4/8		
X-rays & Chl. (1000 μg/ml)	5/9, 4/8 3/8, 6/8 3/8, 3/8 3/10, 3/7 4.5/9, 3/7 6/7, 3/9 3/8, 3/8 4/8, 3/7 0/8, 4/7 3/8,	1/6 5/6 3/7 4/6 3/6 3/6	3/6	0/8, 0/6 4/7, 0/8 2/7 0/? 7/8 4/6 2/6 4/8 0.5/10 0.5/10	2/2	0/8, 9/11 4/10, 3/9 3/8, 0/8 2/9, 0/8 5/9.5,6/10 4/8 3/9 2/8 0/9 3/8	2/6 0/4 0/?	2/2
X-rays & Acrif. (2×10^{-4} M)	1/10 3/8 4/9 7/11 4.5/5	2/4						

Chl. = chloramphenicol: Acrif. = acriflavine.

Each fractional number = $\frac{\text{No. of gyres between kinetochore and break point}}{\text{Total no. of gyres of whole arm}}$

TABLE 8. Data on distribution of induced breaks along chromosome arms of *Trillium*. III) Simple iso-chromatid breaks (B″).

Treatments	Chromosome arms							
	A	**Bl**	**Bs**	**Cl**	**Cs**	**Dl**	**El**	**Es**
X-rays	6/10,6/10 4/10,6/8 1/9,3/8 4/7,5/7 2/9,2/7 2/9,5/7 2/10 4/10 3/7.5 3/9	3/5 1/6 0/? 7/9 2/6 2/6 2/6 2/6	0/4	6/8 7/9 6/8 6/9 4/8 4/8 1/8	0/3 0/3	7/9 5/9.5 7/9 8/10 9/12 7/10 4/10 1/11 5/9 4/7	0/? 2/5	
X-rays & Chl. (100 μg/ml)	0/? 3/11 4/7 4/8	2/7 2.5/6.5 3/7 2/6.5	2/4 2/6	2/7 3/8 8/11 2/6.5 3/8 2/9		4/9 9/11 4/11 8/10 6/12 7/8	3/5	
X-rays & Chl. (500 μg/ml)	2/8 3/7 2/9 4/9 3/9 3/8 7.5/9.5 2/8 6/9 4/6	5/7 1.5/7 3/7		3/8 2/8 2/8 8/10 2/8 6/8 5/9 2/9 3/8 4/8		7/10 6/10 6/10		
X-rays & Chl. (1000 μg/ml)	3/9,4/8 5/7,2/9 0/9,7/9 3/9,4/8 1/7,8/9 2/9,2/8 4/8,6/8 3/8,4/9 4/8,0/? 4/8,4/8 4/6, 3.5/8 3.5/8, 2/8 3.5/8, 7/8 6.5/8	2/5 2/6 5/7 2/4 2/5 3.5/5 0/6 3/6 3/7 4/6		4/8 4/8 1/8 7/9 4/7 4/8		6/9 5/11 6/9 6.5/9 2/10 5/10 5.5/9 4/8 7/9 7/9	2/5 0/?	
X-rays & Acrif.(2×10^{-4} M)	4/8 2/9 3/8 1/8 3/9.5	2/5 3/4 1/7		4/8 6/8		9/10 6/10 8/10		1/3

Chl. = chloramphenicol: Acrif. = acriflavine.

Each fractional number = $\frac{\text{No. of gyres between kinetochore and break point}}{\text{Total no. of gyres of whole arm}}$

TABLE 9. Data on distribution of induced breaks along chromosome arms of *Trillium*. IV) Rejoined iso-chromatid breaks (rB″)

Treatments	Chromosome arms							
	A	**Bl**	**Bs**	**Cl**	**Cs**	**Dl**	**El**	**Es**
X-rays				6/8				
X-rays & Chl. (500 μg/ml)				4/8		4/10		
X-rays & Chl. (1000 μg/ml)	1/8			4/7				
	6.5/8			4/8				
	6.5/8							
	4/6							
	3/6							
	4/8							
	4/8							

Chl. = chloramphenicol.

Each fractional number = $\frac{\text{No. of gyres between kinetochore and break point}}{\text{Total no. of gyres of whole arm}}$

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Summary

1) Modifying effect of chloramphenicol on the frequency of X-ray-induced chromosome aberrations was investigated with the PMCs of *Trillium kamtschaticum* PALL.

2) Two-hit aberration yield increases to a certain degree by the combined chloramphenicol treatment.

3) From the comparison of the frequencies and types of rejoined breaks and of simple iso-chromatid breaks in each set of the treatments, it is supposed that some of iso-chromatid breaks in the case of simple irradiation convert, when combined with chloramphenicol treatments, to the chromatid breaks which will result in chromatid-chromatid or chromatid-chromosome interchanges.

4) The alteration of the types and frequencies of the aberrations by the combined chemical treatments was explained on the basis of the inhibition effect of chloramphenicol on the rejoining of broken ends, that is, the prolongation of their open-periods and following joinable periods.

5) As to the distribution of the induced breaks in each chromosome, it was found that the frequency of simple iso-chromatid breaks seems to be less in **B** and **E** chromosomes in both the cases of the simple irradiation and the combination treatments.

6) A tentative experiment utilizing acriflavine indicated that any effect of the modification was not remarkable, in so far as its concentration at 2×10^{-4} Mol is concerned.

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Explanation of Plates

Plate I

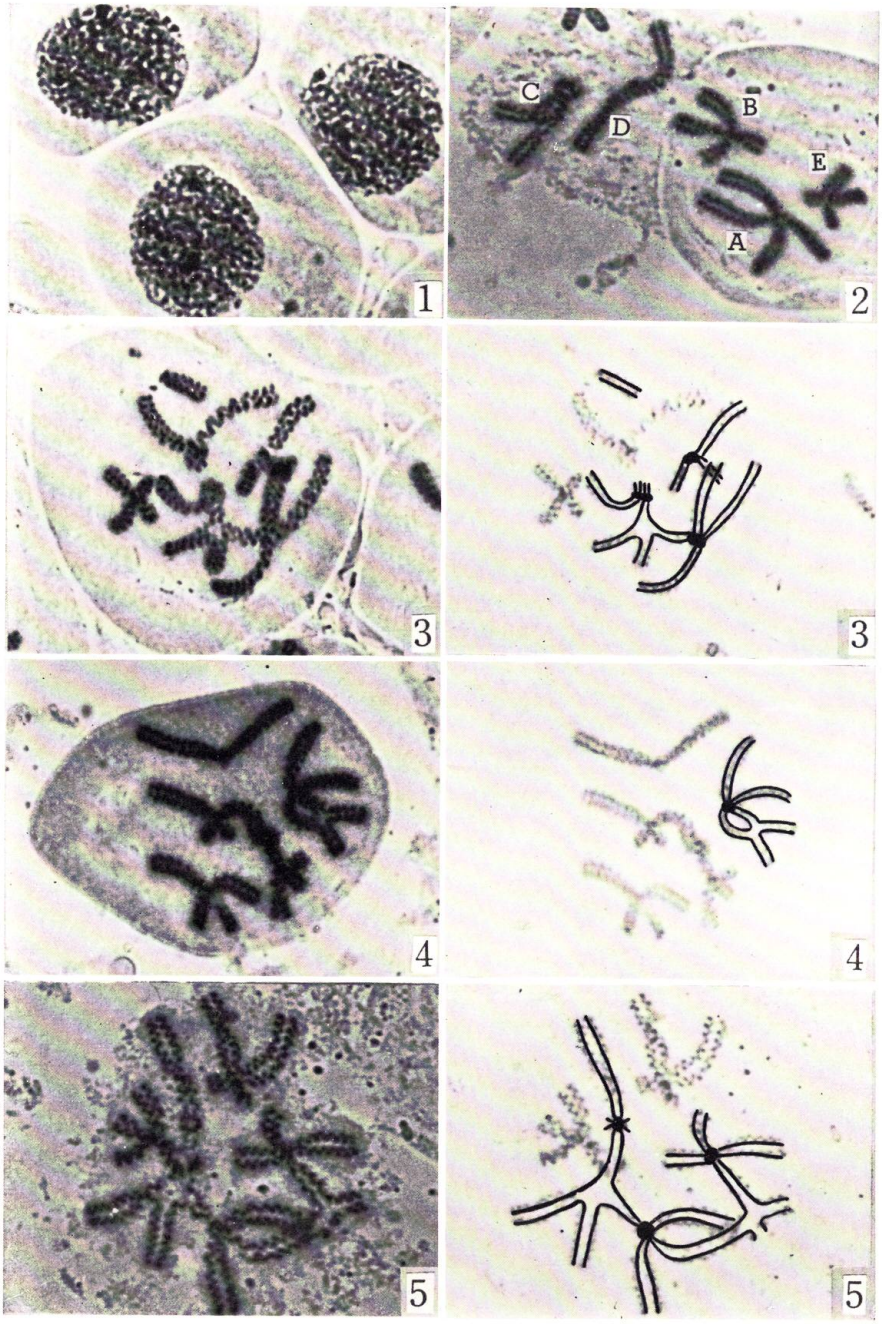
Fig. 1. Early prophase cells at the beginning of treatments (about 10 days before 1st metaphase).

Fig. 2. A complete set of chromosomes in the chemical control material.

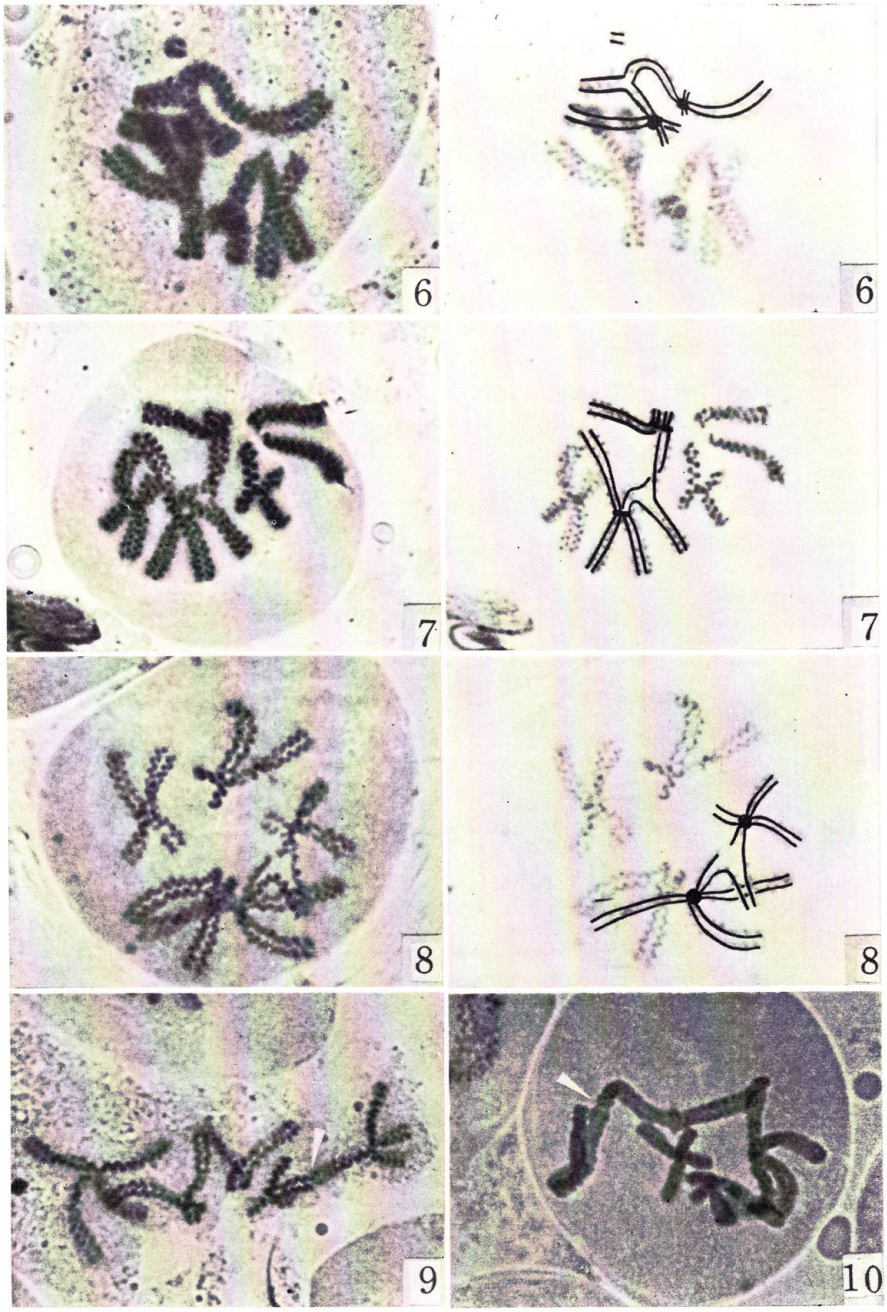
Figs. 3–5. Various types of aberrations at 1st metaphase in the X-rayed materials. 3, iso-chromatid breakage in **B**-chromosome (B″) and chromatid-chromatid interchange (T″) between **A**- and **C**-chromosomes. 4, intrabivalent T″ in **A**-bivalent. 5, T″-s between **A**- and **D**- chromosomes, and between **A**- and **B**-chromosomes.

Plate II

Figs. 6–10. Various types of aberrations at 1st metaphase in the X-rayed materials. 6, chromosome-chromatid interchange between **C**- and **D**-chromosomes (T_1''). 7, union between unbroken chromatid ends of **C**-chromosome and two broken ends of a chromatid of **A**-chromosome (U′). 8, union between an unbroken chromatid end of **E**-chromosome and a broken end of chromatid of **A**-chromosome (U′). 9 & 10, unions between two unbroken ends of different chromosomes (U^0). U^0 between **B**- and **E**-chromosomes in figure 9, and U^0 between **B**- and **D**-chromosomes in figure 10.



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