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Changes of X-ray sensitivity of *Trillium* chromosome during meiosis, with special reference to effects of nitrogen gas and carbon monoxide on the sensitivity

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Introduction

It is well known that the radiation sensitivity of cells varies with stages of the division cycle. The sensitivity of chromosomes at any stage is usually estimated from the yield of chromosome aberrations revealed at certain division stage such as metaphase or anaphase. However, there is much

disagreement concerning the change of the radiation sensitivity during cell division cycle. SAX (1940) and CREIGHTON (1941) have found that prophase was most sensitive. NEWCOMBE (1942) observed that the sensitivity increased as the chromosomes approached to metaphase in pollen of *Tulipa* and *Hyacinthus*. Some authors found two stages of high sensitivity, *e.g.*, metaphase and anaphase in pollen of *Tradescantia* (BISHOP 1950), mid-prophase and mid-telophase in neuroblast of *Chortophaga* (AMAND 1956) and metaphase and anaphase in *Trillium* (SPARROW 1944, 1951). Such disagreement among the reports may, in part, be due either to an ambiguity inevitable in determining real stages at which radiation was emitted accompanying the delaying effect of radiation on the progress of cell division, or to specificities of material species and tissues.

Various factors modifying chromosome aberrations induced by ionizing radiation have been reported. They are temperature (SAX and ENZMANN 1939, GILES 1954, WOLFF and LUIPPOLD 1955), ultrasonication (CATALDO *et al.*, 1973), ultraviolet radiation (SCHULTZ 1951, SWAMINATHAN and NATARAJAN 1959), water content (CONGER *et al.* 1966, AHNSTRÖM and SANNER 1971), centrifugation (ANDERSON 1955) and numerous chemical agents containing respiratory inhibitors such as KCN and CO (WOLFF and LUIPPOLD 1955, KIHLMAN 1958, 1963).

The oxygen tension is the most effective one among factors affecting the radiosensitivity which is represented as chromosome aberrations. For example, the frequency of chromosome aberrations induced under anoxia is almost one half or a third of the frequency by the irradiation in air.

Since the discovery of the oxygen effect by THODAY and READ (1947) many evidences on the oxygen effect have been accumulated. It is known also that chromatid deletions are less affected by anoxia than iso-chromatid breaks and chromatid exchanges (RILEY *et al.* 1952, SWANSON and SCHWARZ 1953). Concerning the oxygen effect on the chromosome aberrations induced by ionizing radiation one hypothesis (GILES *et al.* 1952, RILEY *et al.* 1952) has been proposed that a breakage mechanism is influenced and the other hypothesis (SWANSON and SCHWARZ 1953) has been postulated that anoxia is in favour of rejoinability of the broken ends of chromosome. Other researchers have also tried to solve this problem (CONGER 1955, NEARY and EVANS 1958).

Because of the restriction of suitable plants for material, cytological consequences of the irradiation revealed during meiosis have been less studied in comparison with those in mitotic cells. Until now the oxygen effect on the X-ray sensitivity of cells during meiotic division has not been investigated.

An aim of the present research is to find out changes of radiation sensitivity of chromosomes during the division cycle of microsporogenesis and pollen mitosis. Moreover, modifying effects of nitrogen gas and carbon monoxide at different stages of meiosis were tested in order to study the nature of changes of the sensitivity of chromosomes to radiation and the ability of restitution.

Materials and Methods

Flower buds of *Trillium kamtschaticum* PALL. collected at Nakagoya in Hokkaido were used as materials throughout the present experiment.

X-ray irradiation was given with 30 R or 50 R at dose rate of 10 R per minute using Toshiba therapy tube operated at 160 KVP mainly and 140 KVP partly. Total dose was compensated automatically by Rhadocon dosimeter.

The rhizomes irradiated were planted in pots and replaced under the natural condition. In some cases they were kept in a constant temperature room at 4 ± 0.5°C or 11 ± 0.5°C.

Cells irradiated at meiotic prophase were scored for aberration at the first metaphase and anaphase, at interphase and at metaphase of microspore division. Scoring of irradiated cells at the first metaphase and subsequent stages was made at microspore metaphase.

In nature, the meiosis of microsporocytes of *Trillium* proceeds very slowly under snow.

Trillium kamtschaticum has an advantage for the cytological researches such as the present investigation in addition to the long size and small number of chromosomes. The meiotic course of *Trillium* microsporocytes takes six months from October to late March at Sapporo. In *Trillium*, each stage of meiosis has considerably long duration, and therefore, pollen mother cells (PMCs) at desired stage can be easily obtained with high synchronization, being more favourable than the estimation of division stages by means of the bud length (MITRA 1958, LAWRENCE 1961 in *Lilium longiflorum*) or the bud location (ECOCHARD 1966 in *Vicia faba*). Gas exchange and chemical and other treatments can be carried out easily depending upon the long period of meiotic cycle and high synchronization of stages.

In the present experiment, treated plants were kept under completely the same condition as control plants except the period of treatment.

Details of the determination of division stage when the PMCs were irradiated, and the design of X-ray irradiation and gas treatments will be explained below when the results of experiments will be described.

Results and Discussion

I. Changes of radiosensitivity during meiotic cycle and effects of nitrogen treatment

The radiation sensitivity at different stages and its modification by nitrogen were examined throughout meiotic cycle.

X-ray irradiation: X-rays were given with 30 R at dose rate of 10 R per minute from Toshiba therapy tube operated at 160 KVP and 3 mA with 0.5 mm Cu and 1.0 mm Al filtration added. In part the operation was made at 140 KVP and 25 mA with 0.3 mm Cu and 0.5 mm Al filters. When PMCs were at three different stages of prophase, *i. e.* zygotene, pachytene and mid-diplotene, flower buds were irradiated in air or under anoxia (in nitrogen). In the other experiment PMCs at each stage from late prophase to tetrad were exposed to 30 R at 4.5 R per minute (150 KVP, 3 mA, 0.5 mm Cu and 1.0 mm Al filters) at 13–17°C.

Nitrogen treatment: For the anaerobic condition a glass chamber (1 liter volume) or a vacuum desiccator (2 liters) in which five plants were kept was evacuated gently with the vacuum pump for few minutes before gas was introduced. The nitrogen was added until the pressure was normal. When this gas exchange were repeated three times, nitrogen gas with purity of 99.9% filled the chamber. In this state plants were kept for 24 hours or 48 hours at 6 ± 1°C for X-ray irradiation at prophase and for 12 hours at 4 ± 0.5°C for irradiation at subsequent meiotic stages. Nitrogen was removed 6.5 hours after irradiation and plants were potted and replaced under the natural condition.

In late February when one or three months elapsed after irradiation PMCs at the first metaphase or anaphase were prepared by 45% acetocarmine smear method. In early April smear preparations were made also with microspores at interphase and metaphase. Scoring of aberrations was done with about 200 cells per individual in four or five plants in each treatment.

1. Preliminary tests.

Before the experiment of X-ray irradiation, it was examined whether there were any changes of the cell division and the chromosome behavior by the reduction of the atmospheric pressure. Rhizomes were put into a vacuum desiccator in which pressure was reduced and irradiated with 30 R X-rays.

In PMCs under reduced pressure cell division proceeded regularly. Cytomixis was not observed and stickiness of chromosomes was not recognizable.

As shown in Table 1 and Figure 1, however, half-chromatid bridges occurred notably which are a characteristic aberration type induced by irradiation at late prophase. Exchange breaks might be influenced most largely if the reduced pressure is affecting to X-ray induced chromosome aberrations. Under normal pressure and reduced pressure, frequencies of half-chromatid bridges were 6.0% and 6.3% and those of chromatid bridges 6.9% and 5.4% respectively. The frequency of the total yield of two exchange types under reduced pressure (11.7%) was not lower significantly than that (12.9%) of control (normal pressure) as well as the frequency of total anomalous cells.

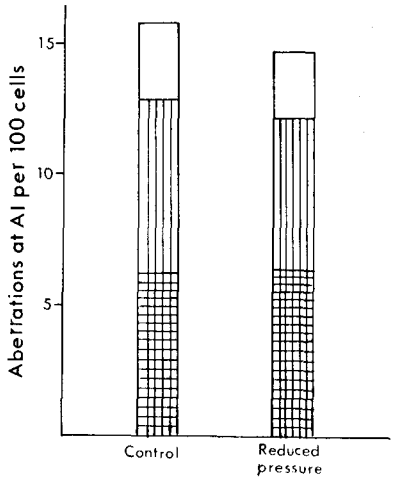


Figure 1. The yield of X-ray induced chromosome aberaations at the first anaphase under reduced atmospheric pressure.
▩: half-chromrtid bridge.
▨: bridge and an accompanying fragment.
▭: acentric fragment and others.

BERG *et al.* (1965) reported the occurrence of chromosome aberrations with higher frequency in barley seeds under oxygen or nitrogen pressure ranging from 500 to 2000 lb/in. Oxygen, at pressure ranging from one to 60 atm, has been considered as a weak mutagene in several materials including *Tradescantia* pollen and *Vicia faba* seeds (CONGER and FAIRCHILD 1952, MOUTSCHEN *et al.* 1959). On the contrary there are no evidences on the effect of reduced atmospheric pressure on chromosome aberrations.

Above results will allow to program the gas treatment without any consideration on the influence of reduced pressure.

TABLE 1. Effect of reduced atmospheric pressure during X-raying revealed as the chromosome aberrations at the first anaphase

	No. of cells observed	No. of half-chromatid bridges	No. of bridges with fragments	No. of acentric fragments	No. of the other aberrations	Total no. of aberrations	Frequency per 100 cells
Control	1929	116	134	23	33	306	15.9
X-rayed under reduced pressure	1442	91	79	16	19	205	14.2

Effect of the lack of oxygen: STEINITZ (1944) observed aberrations by nitrogen in microsporocytes of *Tradescantia*, such as the lengthening of the period of prophase and interphase and the formation of chromosome fragments, bridges and lagging chromosomes. The time duration of nitrogen treatment in his experiment was 12 hours.

In the present experiment cells at diplotene stage were treated with nitrogen for 24 hours. The first metaphase cells one week after gas treatment were quite normal and showed no appreciable aberration in chromomosomes. In Steinitz's experiment temperature (23°C) may have some relation to the effect on chromosomes.

2. Chromosome aberrations induced by X-ray irradiation at prophase.

PMCs were irradiated with 30 R at three different stages of prophase: zygotene, pachytene and mid-diplotene (cf. Plate I). And then, they were observed at the first metaphase. Aberrations of simple breakage were classified as iso-chromatid breaks (B″), chromatid breaks (B′) and minute acentric fragments. Aberrations of exchange types scored at the first metaphase were chromatid translocation (T″), chromatid-chromosome translocation (T‴) and chromosome translocation (T⁗). Microphotographs of these aberrations are shown in Plate III.

Results are presented in Table 2. Iso-chromatid breaks were yielded with the highest frequency occupying about 72 per cent of total aberrations in each stage. At all stages both simple breaks and aberrations of exchange types occurred. Among three stages cells at pachytene had significantly higher radiosensitivity of chromosome than those at the other two stages which showed very similar sensitivity to each other representing similar frequency of iso-chromatid breaks and total simple breaks. It seems that the aberration of exchange types is higher at pachytene, since the frequencies of aberration of these types at zygotene, pachytene and mid-diplotene were 7.0, 9.7 and 8.7%, respectively.

3. Effect of nitrogen on the yield of chromosome aberrations.

In parallel with the above observation of PMCs X-rayed in air, effects of nitrogen on X-ray induced chromosome aberrations were examined. X-ray irradiation of 30 R was given to the plants under nitrogen treatment which had started 14 hours before irradiation. Plants treated with nitrogen and X-ray were kept under the same condition as the plants simply rayed in air throughout this experiment. Control plants were also put into a desiccator and evacuated with a vacuum pump. Nitrogen pressure was one atm.

Results are shown in Table 3. When rhizomes were kept in nitrogen for 24 hours, frequencies of iso-chromatid breaks were 12.9%, 7.5% and 9.9% for observed cells at zygotene, pachytene and diplotene respectively, while aberrations of exchange types occurred with the same frequency among three stages. Keeping rhizomes in nitrogen for 48 hours caused far less aberrations in PMCs irradiated at all three stages.

Nitrogen treatment showed fairly strong effects on the reduction of the yield of aberrations (cf. Tables 2 and 3, Figures 2, 3 and 4). Among the plants irradiated in air the yield of aberrations was higher in the case irradiated at pachytene and among the plants treated by nitrogen the aberration fre-

TABLE 2. Frequencies of chromosome aberrations at the first metaphase following X-ray irradiation at different stages of prophase

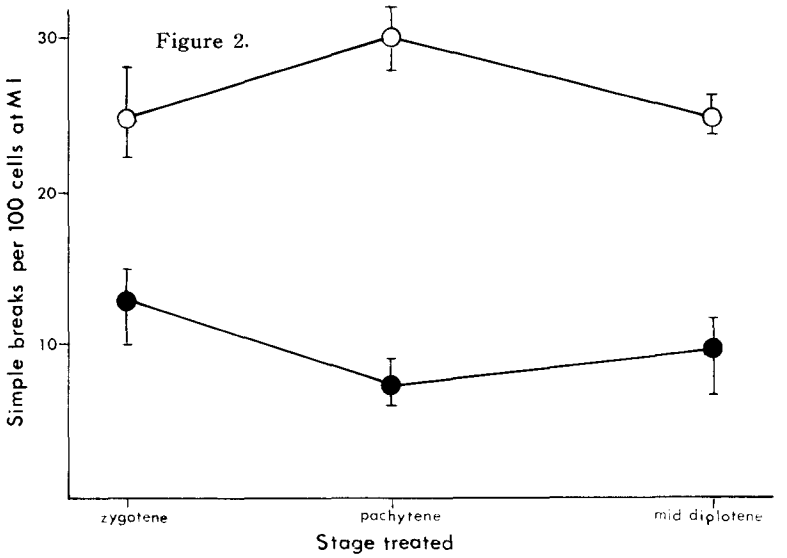
Stage	Treatment	No. of cells observed (plants)	Aberrations*					Frequency (per 100 cells)	
			B″	B′	T″	T‴	T⁗	Simple breaks	Exchanges
Zygotene	X-ray	1055(5)	264	20	61	17	2	25.02(±1.37)**	7.01
Pachytene	"	850(4)	256	10	70	10	3	30.12(±1.98)	9.76
Mid-diplotene	"	565(4)	142	6	60	8	1	25.13(±1.12)	8.67

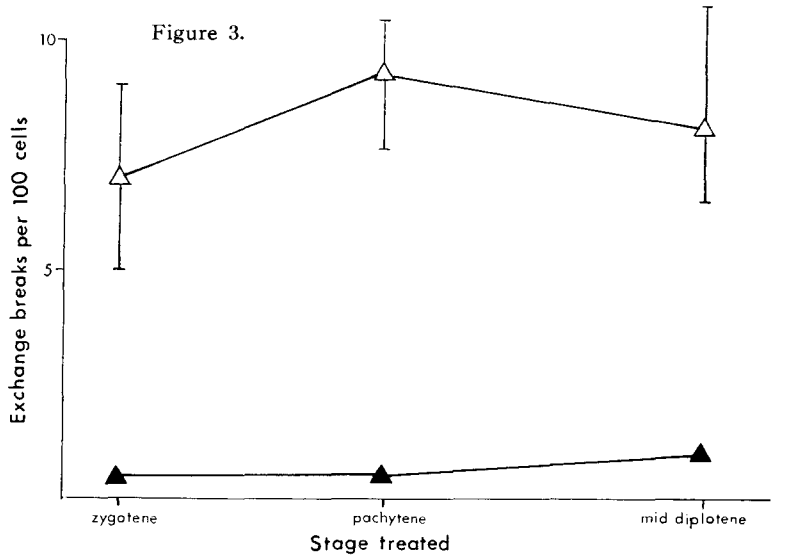
*: B″, B′, T″, T‴ and T⁗ represent simple iso-chromatid breaks, acentric chromatid fragments, chromatid translocation, chromatid-chromosome translocation and chromosome translocations, respectively.
**: (±S. E.)

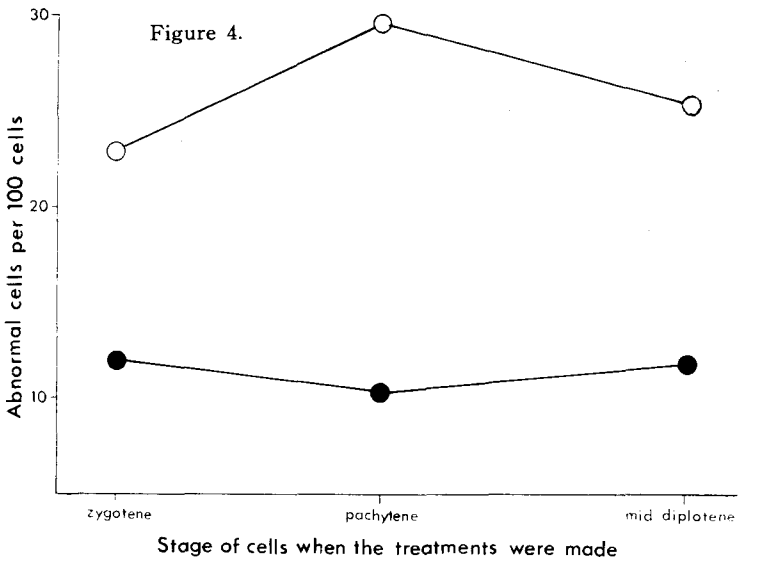
TABLE 3. Frequencies of chromosome aberrations at the first metaphase following X-ray irradiation and the combined nitrogen gas treatment at different stages of prophase

Stage	Treatment	No. of cells observed (plants)	Aberrations					Frequency (per 100 cells)	
			B″	B′	T″	T‴	T⁗	Simple breaks	Exchange
Zygotene	N_2-1*+X-ray	1000(5)	129	11	5			12.90(±1.07)	0.50
Pachytene	N_2-1+X-ray	1000(5)	75	4	5			7.50(±0.21)	0.50
	N_2-2+X-ray	800(4)	44	4	2	1		5.50	0.38
Mid-diplotene	N_2-1+X-ray	1096(5)	108	2	6	1		9.85(±1.11)	0.64
	N_2-2+X-ray	791(4)	47	1	3	1		5.94	0.51

*: N_2-1=24 hrs. in nitrogen gas.
N_2-2=48 hrs. in nitrogen gas.







Figures 2, 3 and 4. Frequencies of chromosome aberrations at the first metaphase following X-ray irradiation and the combined nitrogen gas treatment at different stages of prophase. Fig. 2. Simple breaks. Fig. 3. Exchange breaks. Fig. 4. Total yield of aberrations.
Open circle: X-ray in air. Closed circle: X-ray in nitrogen.

quency was lower in the plants irradiated at pachytene. Namely, the extent of the reduction by nitrogen was the largest at pachytene. The ratio of the aberration frequency of iso-chromatid breaks in the plants irradiated in air and in nitrogen was 4.0 at pachytene, 2.5 at zygotene and 2.55 at mid-diplotene, respectively. Aberrations of exchange types were also reduced remarkably by nitrogen.

The ratio of aberrations obtained in air and anoxia (nitrogen) has been used as an index of the effect of oxygen. The value of the ratio has been reported to be about 2.5 to 3.0 from previous works (THODAY and READ 1947, NEARY and EVANS 1958). The value of 4.0 at pachytene in *Trillium* PMCs exceeds above-mentioned values. In ascites tumor cells the value of 3.7 was reported by Howard-Flanders and Moore (1958).

In order to examine chromosome aberrations at the first anaphase cells at that stage were scored for aberrations. Some plants used for the examination at the first anaphase were not same individuals as used in the observation at the first metaphase. The comparison of the radiosensitivity among three stages was not difficult since observed aberration types were not different with each other (Tables 4 and 5). From the results presented in Tables 4 and 5 one can find that the pattern of the change in radio-

TABLE 4. Numbers of aberrant cells at the first anaphase following X-ray irradiation in air at different stages of prophase

Treated stages	No. of cells observed (plants)	No. of cells with						No. of total fragments	Total abnormal cells	
		br+f	br	br+2f	hbr	1f	2f		Numbers	Frequencies (%)
Zygotene	1944(5)	261	84	41	0	268	31	686	693	35.6
Pachytene	2163(4)	294	76	88	0	487	125	1246	1060	49.0
Mid-diplotene	1668(4)	239	109	15	28	227	122	530	688	41.2

br+f: cell possessing one bridge and an accompanying fragment.
hbr: half-chromatid bridge.

TABLE 5. Numbers of aberrant cells at the first anaphase following X-ray irradiation in nitrogen at different stages at prophase

Treated stages	No. of cells observed (plants)	No. of cells with						No. of total fragments	Total abnormal cells	
		br+f	br	br+2f	hbr	1f	2f		Numbers	Frequencies (%)
Zygotene	1923(5)	83	30	11	0	128	16	369	271	14.1
Pachytene	2449(5)	89	58	7	1	129	17	262	263	10.7
Mid-diplotene	2451(4)	123	85	6	1	120	8	270	333	13.6

TABLE 6. Distribution of iso-chromatid breaks in the chromosome arms

Treatments		Chromosome arms										
		A	A	Bl	Bs	Cl	Cs	Dl	Ds	El	Es	Total
X-ray in air	Observed no.	111	111	49	23	144	0	204	0	9	11	662
	Expected no.	98.5	98.5	84	50	100	28	103	6	58	36	662
X-ray in nitrogen	Observed no.	57.5	57.5	29	14	58	1	76	0	11	4	312
	Expected no.	46	46	40	23	47	13	49	3	28	17	312

Expected no.: Numbers calculated as proportional to the arm length which were estimated by the number of gyres of each arm.

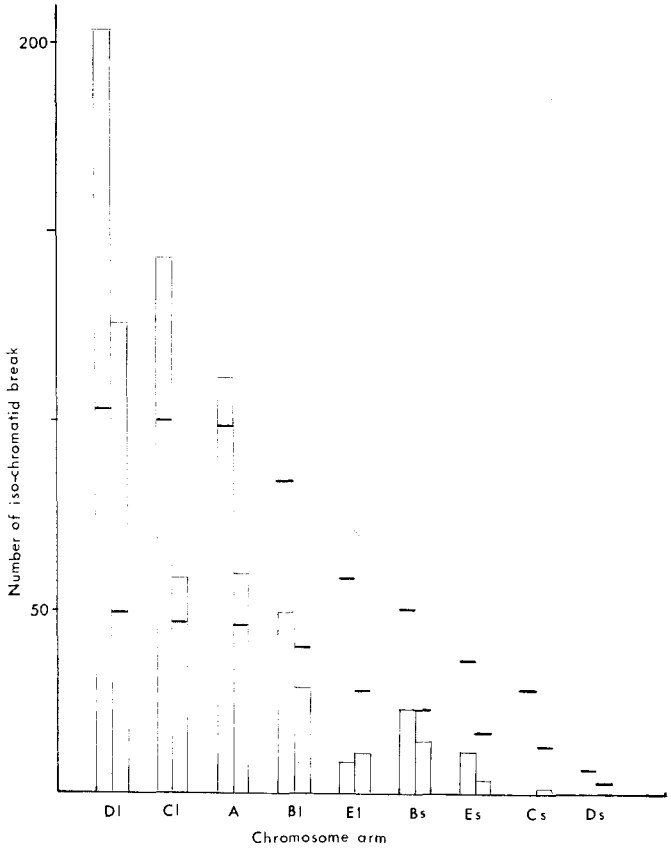


Figure 5. Distribution of iso-chromatid breaks in chromosome arms. Right and left columns of each arm represent breaks in air and in nitrogen respectively. Horizontal lines represent the expected number calculated as proportional to the arm length.

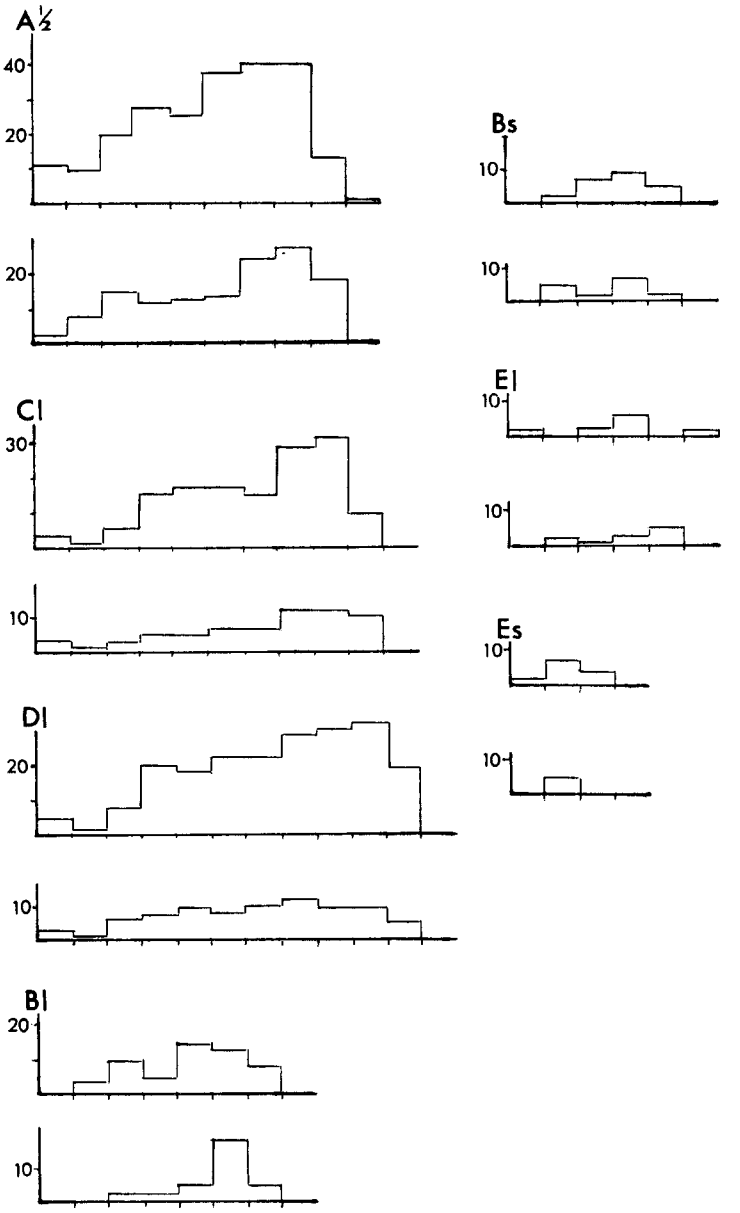


Figure 6. Distribution histogram of iso-chromatid breaks along chromosome arms. In each arm the upper graph shows the result obtained from the treatment in air and the lower graph in nitrogen. Abscissa represents the arm length by gyre numbers.

sensitivity among three stages is very similar to that observed at the first metaphase.

4. Distribution of breaks.

The distribution of 662 iso-chromatid breaks obtained in air and 312 in nitrogen, which were observed at the first metaphase in the above experiments, was analysed over five chromosomes (A, B, C, D and E). The distance to a break point from the kinetochore was represented by the gyre number of the chromosome spiral structure (cf. Plate I–5).

Table 6 and Figure 5 show the distribution of isochromatid breaks produced both in air and nitrogen. Long arm of D chromosome in air yielded more breaks and in B and E breaks occurred a little less.

The spacial distribution over various regions along the length of chromosome arm was estimated with the supposition of disclosure of labile sites of the chromosome for nitrogen treatment. As shown in Figure 6 the distribution pattern of breaks produced in nitrogen is similar to that in air within each chromosome arm. And in both air and nitrogen the majority of breaks occurred at the mid-arm region near the distal end.

MATSUURA and TANIFUJI (1962) reported that in B and E chromosome simple chromatid breaks were less in the cases of X-ray irradiation and of combination treatment with chloramphenicol. The less frequency of breaks in B chromosome shown in the present observations is parallel with their result.

5. Setting of anomalous type as an index.

For the determination of radiosensitivity at different stages of meiotic division cycle the observation at microspore division was required. The problems which should be solved were as follows. Is there a correlation between chromosome breakages induced by irradiation and anomalous types observed at the subsequent stages of meiosis and microspore division? Which is available as an index among micronuclei, fragments at the metaphase of microspore division, and dwarf pollen grains?

Acentric fragments at the first metaphase and anaphase conceal themselves into the daughter nuclei at tetrad stage or transform into micronuclei at that stage. When the tetrads break up to microspores, micronuclei either co-exist with major nucleus in any one cell or give rise to small extra-microspores. Some of the latters develop into dwarf pollen (cf. Plates VI and VII). Lagging chromosome would also be expected to form micronuclei. However, since the number of lagging chromosome is very small, their contribution to

micronuclei is negligible.

Trillium buds which had PMCs at late prophase were irradiated by X-rays (30 R) in air and in nitrogen. Chromosome aberrations were scored at the first metaphase and subsequent stages of meiosis. The anomalous cell types at the first metaphase and anaphase were relatively simple but aberrant cells at the second division were more complex, containing newly appeared

TABLE 7. Frequencies of chromosome aberrations at successive stages of meiosis following X-ray irradiation (30 R) at late diplotene

Stage observed	Treatment	No. of cells observed	No. of iso-chromatid breaks	No. of chromatid breaks	No. of minute fragments	No. of exchange chromatid (T'')	Frequency of aberrant cells (%)
MI	N^2–X	592	50	21	11	15	17.4
	X	425	51	13	2	26	23.7

			No. of cells with						Total no. of fragments	Frequency of fragmentation (%)
			br+f	br+2f	hbr	1f	2f	reserved mn		
AI	N_2–X	510	61	7	1	76	4		176	34.5
	X	1264	115	29	23	282	36		561	44.5
TI	N_2–X	2651	203	28	45	427	93	74	917	34.6
	X	495	98	7	2	71	8		210	42.4

Reserved mn: Fragments whose development into micronuclei is expected.

(continued)

Stage observed	Treatment	No. of cells observed	No. of cells with						Total no. of		Frequency of fragmentation (%)
			br+f	cross br+f	1f	1f in a daughter cell	micronucleus	reserved mn	f	mn	
Interkinesis	N_2–X	1537	50		129	43	38	45	234	112	21.9
	X	475	36		31	41	23	38	72	93	33.9
MII	N_2–X	777	13	16	74	55	31	40	230	99	42.3
	X	311	2	19	3	44	28	22	96	66	52.0
AII	N_2–X	701	39	8	76	31	19	14	221	38	36.8
	X	1149	37	27	115		58	105	299	210	44.3
TII	X	493	10	10	37	55	24	12	156	50	41.9
Tetrad	N_2–X	230			4		34		4	54	25.2

Main types of aberrant cells are given in this table.
Cross br+f: bridges crossing through a septum between daughter cells.
1f in a daughter cell: one fragment in one daughter cell and no fragment in the other. f: fragment. mn: micronucleus.

TABLE 8. Frequencies of micronuclei and dwarf grains at the interphase and fragments at the metaphase of microspore division following X-ray irradiation at the microsporocyte prophase

Treatment	No. of cells observed	Micronuclei		No. of cells observed	Dwarf grains		No. of cells observed	Fragments	
		Number	Frequency (%)		Number	Frequency (%)		Number	Frequency (%)
X-ray in air	4117	253	6.1	10328	786	7.6	117	18	15.4
X-ray in nitrogen	1846	87	4.7	5240	282	5.4	339	40	11.8
Without treatment	3066	3	0.1	9034	48	0.5	379	2	0.5

TABLE 9. Frequencies of dwarf grains and polyploid cells at the microspore metaphase following X-ray irradiation at three different stages of microsporocyte prophase

Stage	X-ray in nitrogen				X-ray in air				Factor (Air/N_2)	
	No. of cells observed	Frequency (%)			No. of cells observed	Frequency (%)				
		Dwarf grain	Complete polyploid cell	Incomplete polyploid cell		Dwarf grain	Complete polyploid cell	Incomplete polyploid cell	Dwarf grain	Polyploid cell
Zygotene	5444	4.09	0.24	0.28	5445	5.52	0.07	0.01	1.35	0.15
Early Pachytene	5440	6.10	0.03	0.25	5762	7.38	0.07	0.24	1.21	1.10
Mid-diplotene	7011	0.58	8.41	0.70	5696	3.52	13.61	0.89	6.07	1.58

bridges, fragments and micronuclei as shown in Plates IV and V. Thus, in Table 7 only main types of anomalous cells were given.

As the results shown in Table 7 and Figure 7 frequencies of total fragments were relatively similar among the first anaphase, the first telophase, the second anaphase and the second telophase-tetrad while they were lower at interkinesis and higher at the second anaphase. The aberration type at tetrad stage was restricted to only one type of micronuclei, and the proportion of its frequency produced in air to nitrogen was about 1.3 resembling to the general proportion observed at the stages ranging from the first metaphase to the second anaphase.

Independent of the above examination the fate of PMCs irradiated by X-rays with or without nitrogen was examined at microspore division. Aberrations were scored concerning dwarf pollen and micronuclei co-existing with major nuclei at late interphase and prophase and fragments at metaphase of microspore division.

Results are shown in Table 8 and Figure 8. The frequency of each abnormal type in plants without treatment was almost equal to zero showing the very high regularity of meiotic process in *Trillium* plant. The frequency of fragments at metaphase was 15.4% in plants irradiated in air and reduced to 11.8% by X-rays in nitrogen showing the reducing ratio of about a fourth. The phenomena of this reduction and the reducing ratio by nitrogen were

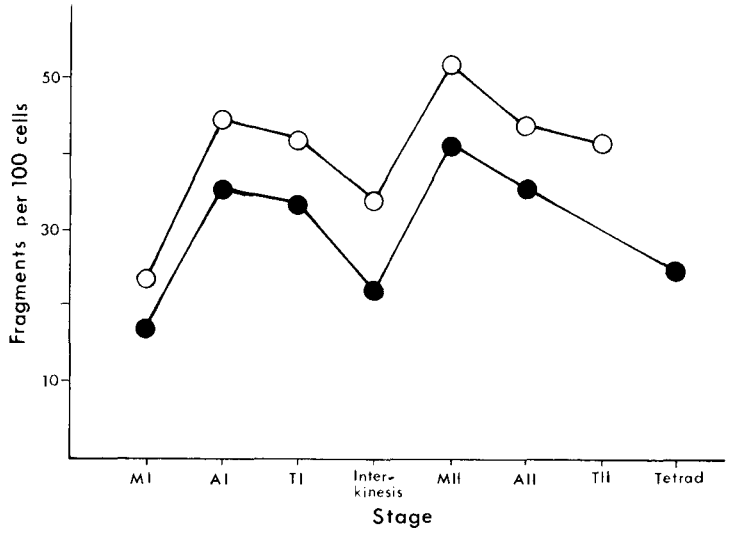
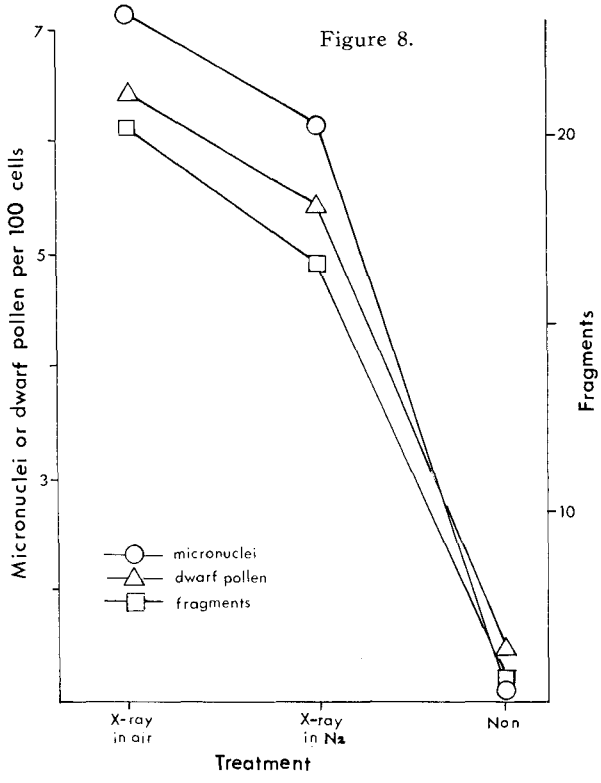


Figure 7. Frequencies of fragments at successive stages of microsporogenesis following X-ray irradiation (30 R) at late diplotene.
Open circle: in air. Closed circle: in nitrogen.



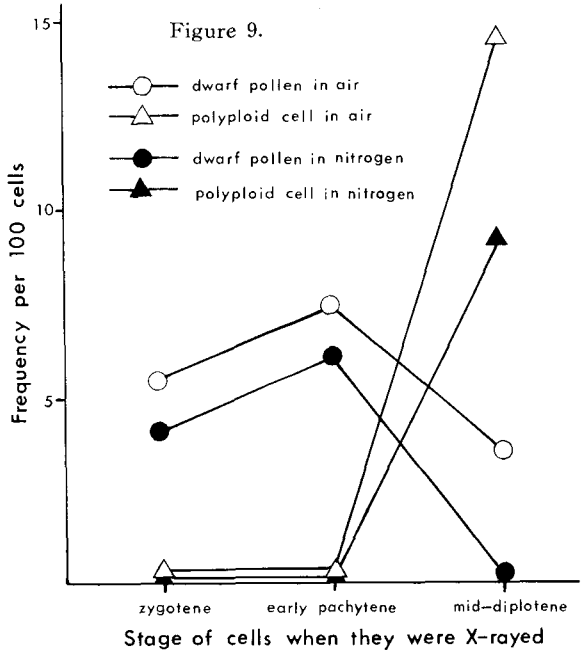


Figure 8. Frequencies of micronuclei and dwarf pollen grains at the interphase and fragments at the metaphase of microspore division following X-ray irradiation at the microsporocyte prophase.

Figure 9. Frequencies of dwarf pollen and polyploid cells at the microspore metaphase following X-ray irradiation at three different stages of microsporocyte prophase.

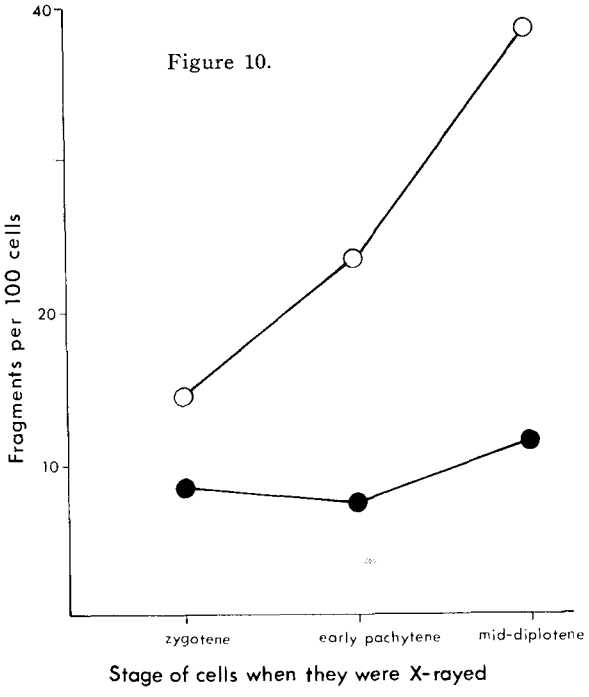


Figure 10. Frequencies of fragments at the microspore metaphase following X-ray irradiation at three different stages of microsporo cyte prophase.

Open circle, -○-: X-ray in air. Closed circle, -●-: X-ray in nitrogen.

similar about dwarf pollen and micronuclei.

It was an interesting finding that the frequency of fragments corresponds to the sum of that of micronuclei and dwarf pollen in both cases irradiated in air and nitrogen. Chromosome fragments at meiotic division conceal themselves into nuclei at microspore interphase or transform to either dwarf pollen or micronuclei. From the present data (cf. Tables 7 and 8, Figures 7 and 8) it is clear that micronuclei co-existing with major nuclei and dwarf pollen as well as fragments at metaphase are able to be used as an index of sensitivity.

In order to estimate the sensitivity of chromosomes at various stages dwarf pollen were scored at microspore interphase following irradiation at different stages of meiotic prophase: zygotene, early pachytene and mid-diplotene. In this examination a sudden occurrence of polyploid pollen in the plants irradiated at mid-diplotene was remarkable in addition to the very low frequency of dwarf pollen by nitrogen treatment at the same stage (Table 9 and Figure 9). Polyploid pollen had round shapes (cf. Plate VII). The occurrence of polyploid cells brings about some confusion to use dwarf pollen as an index.

Table 10 and Figure 10 show the results of the observation of aberrant metaphase microspores following X-ray irradiation (50 R) at zygotene, early pachytene and mid-diplotene. Radiosensitivity of PMCs at mid-diplotene was higher in air and lower in nitrogen than that at zygotene or early pachytene.

ECOCHARD (1966) observed the occurrence of bridges and fragments at the first anaphase and micronuclei at tetrad following X-ray irradiation at leptotene in PMCs of *Vicia faba*. He reported micronuclei at tetrad increased more than direct proportionality with exposure in the range from 25 R to 100 R. SPARROW and SPARROW (1950) studied on spontaneous chromosome breakage in PMCs of *Trillium erectum*. They scored fragments at the first anaphase and metaphase of meiosis and micronuclei at tetrad and microspore interphase. And they observed that micronuclei at microspore interphase were too high frequent to be used as a control count of spontaneous fragmentation. From the present examination the author considers that micronuclei at microspore interphase are reliable as a control count under the condition in which enormous amount of aberrations is produced by irradiation.

6. Radiosensitivity of PMCs at different stages of meiotic division cycle and its modification by nitrogen.

In this experiment *Trillium* PMCs are irradiated at different stages of

TABLE 10. Frequencies of fragments at the microspore metaphase following X-ray irradiation at three different stages of microsporocyte prophase

Stage of treatment	X-ray in nitrogen: No. of cells observed	1f cell	2f cell	Fragments: Total number	Frequency (%)	X-ray in air: No. of cells observed	1f cell	2f cell	Fragments: Total number	Frequency (%)	Factor (Air/N)
Zygotene	359	20		24	8.27	214	19	5	31	14.28	1.75
Early pachytene	315	9	2	22	7.10	129	16	4	30	23.25	3.27
Mid-diplotene	109	7	1	12	11.0	235	40	11	86	38.22	3.47

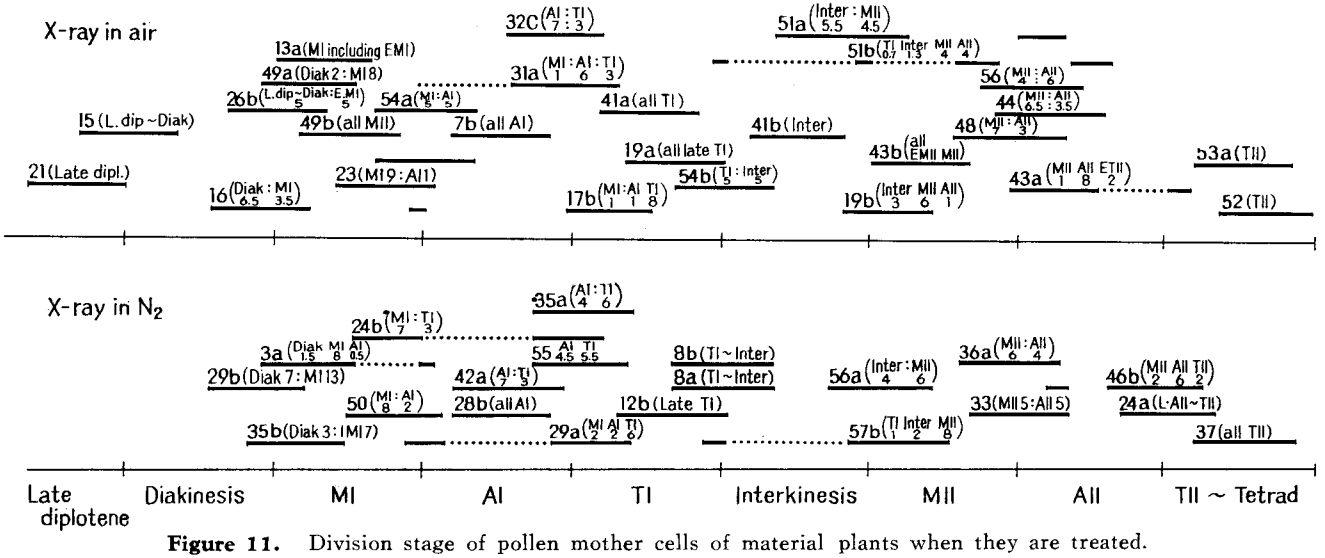


Figure 11. Division stage of pollen mother cells of material plants when they are treated.

TABLE 11. Frequencies of fragments at the microspore metaphase following X-ray irradiation at different stages of microsporogenesis

	X-ray in air	X-ray in nitrogen
Late diplotene — diakinesis	32.1, 48.2	
Diakinesis — first metaphase		9.8, 21.2
First metaphase	70.8, 67.6, 86.1 76.0, 48.0, 65.7	46.1, 45.6, 39.7
First metaphase — first anaphase	84.2, 73.0	
First anaphase	51.6, 56.4, 41.3	44.9, 35.4
First telophase	49.2, 46.9, 38.7	25.6, 22.1, 24.0 14.3
First telophase — interkinesis	31.1	14.2, 8.2
Interkinesis	31.7, 21.2	
Interkinesis — second metaphase		18.3
Second metaphase	41.4, 42.4, 37.3 37.6	32.4
Second metaphase — anaphase	34.3, 22.1	23.0, 25.8
Second anaphase	16.7	26.7, 26.2
Second telophase — tetrad	8.3, 15.6	14.7

Numerals represent the frequency of fragments per 100 cells.

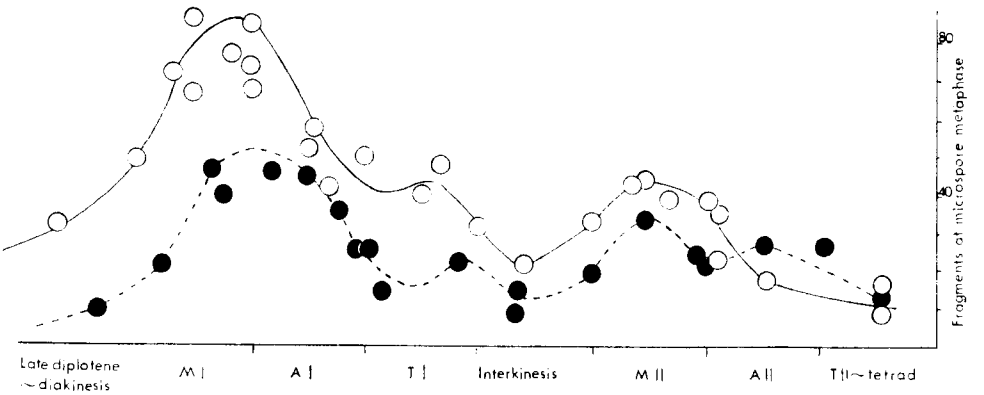


Figure 12. Frequencies of fragments at the microspore metaphase following X-ray irradiation at different stages of microsporogenesis. Open circle and solid line: X-ray in air. Closed circle and broken line: X-ray in nitrogen.

meiotic cycle. X-ray irradiation was given with 50 R at dose rate 4.5 R per minute at 150 KVP and 3 mA at 13°C. The time duration of nitrogen treatment at 4°C was 13.5 hours and 7.5 hours prior to and after irradiation respectively. Immediately after the removal of nitrogen anthers of both irradiated plants and plants without treatment were chosen for determination of the stage. Among buds there appears a difference concerning to the ratio of the first metaphase (MI) and anaphase (AI); and every buds were labelled by that ratio, *e.g.* MI : AI = 8 : 2. It was possible to select plants showing a high synchronization at desired stages. Thus a diagram shown in Figure 11 was made and used to estimate changes of radiosensitivity. Chromosome aberrations were scored at microspore metaphase.

Observed anomalous types were iso-chromatid breaks, chromatid breaks and minute fragments. Diploid cells bearing one or two fragments were observed occasionally. Iso-chromatid breaks were most frequent. The frequency of total fragments was used as the index of radiosensitivity. In Table 11 frequencies at different stages are summarized. Numerals in the table represent frequencies of fragments obtained in individuals whose stages were determined. For example, the numeral 32.1 at late diplotene in air represents the frequency of No. 21 in Figure 11. Thus the radiosensitivity of chromosomes at different stages is shown in Figure 12.

7. Radiosensitivity of post-meiotic interphase of microspore.

Cells at G_1-, S- and G_2-period of post-meiotic interphase were examined on the radiosensitivity of chromosomes. The estimation of stages was carried out by the morphological observation and tritium labelling method. Chromosome fragments induced by X-ray irradiation (50 R) in air and under anoxia were scored.

As shown in Table 12 and Figure 13 G_1 cells were highly sensitive and the sensitivity of G_2 cells was low and hardly affected by anoxia.

The data (Tables 11 and 12, Figures 12 and 13) suggest that the stage with maximum radiosensitivity is at the first metaphase or adjacent stages to it. The sensitivity which increases rapidly to the first metaphase from late prophase, diminishes with the progress to the first anaphase and dwindles by degrees toward tetrad stage while the sensitivity rallies at the second metaphase. The sensitivity of the first metaphase is octuple and that of the second metaphase is quadruple compared with that of tetrad. As regards the evidence of maximum sensitivity of the first metaphase the present results well accord with the observation in *Trillium erectum* by SPARROW (1951).

TABLE 12. Frequencies of chromosome fragments at the microspore metaphase induced by X-ray irradiation (50 R) in air and nitrogen at three stages (G_1, S and G_2) of the microspore interphase

Stage rayed	Treatment	No. of cells observed	Iso-chromatid breaks	Chromatid breaks	Minute fragment	Gap	Total fragments	
							Number	Frequency (%)
G_1	X-ray	529	57	5	10	4	76	14.4
	X-ray in N_2	476	26	1			27	5.7
S	X-ray	532	35	2	4	1	42	7.7
	X-ray in N_2	268	6				6	2.2
G_2	X-ray	1264	59	20	13		92	7.2
	X-ray in N_2	1078	34	16	8	2	60	5.7

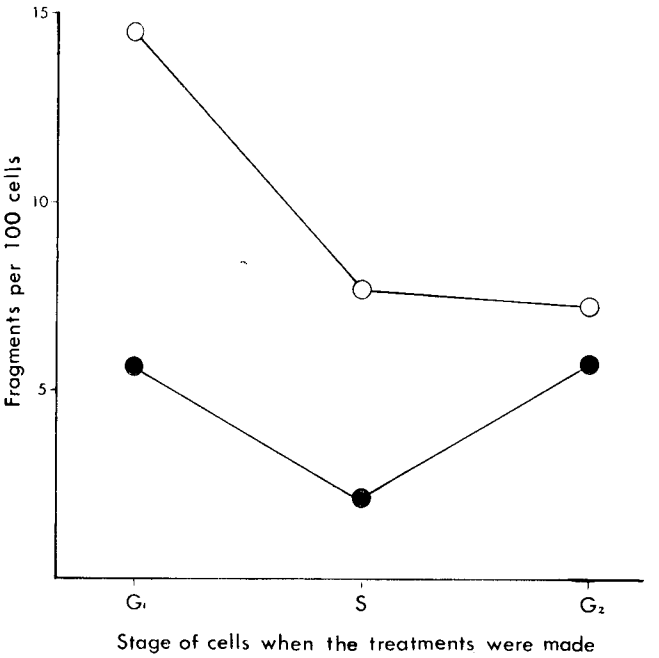


Figure 13. Frequencies of chromosome fragments at the microspore metaphase induced by X-ray irradiation (50 R) in air and nitrogen gas at three stages (G_1, S and G_2) of the microspore interphase. -○-: X-ray in air. -●-: X-ray in nitrogen.

According to his calculation, the relative sensitivity showed an increase from 1 at leptotene to 5.9 at the first metaphase. Experiments on X-ray irradiation at different stages of prophase lead to estimate changes of the sensitivity from leptotene to mid-diplotene. When pachytene stage is divided to early-, mid- and late-pachytene, it is likely that the sensitivity drops down at mid-pachytene and ascends at late pachytene.

Nitrogen during X-raying affected the sensitivity at all stages of microsporogenesis except the second anaphase. The curve of nitrogen in Figure 12 appears to shift to the right as a miniature copy of the curve of air. When a provisional calculation is made based on changes of the sensitivity in Table 12 the ratio of air to nitrogen is 3.25 at diakinesis, 2.3 at the first metaphase which bears maximum sensitivity in air and 1.4–2.0 at other stages except 1.2 at the first telophase. It is suggested that the modification effect of nitrogen may be prominent at prophase (cf. Tables 3 and 9). Taking account of these results, changes of radiosensitivity and the modification by nitrogen at different stages of meiotic cycles are shown in Figure 14.

HOTTA *et al.* (1965) and HOTTA and STERN (1971) found out the satellite synthesis of DNA at pachytene of *Lilium* meiosis. They also reported the endogenous nicking and repair of DNA scission at pachytene and pairing, chiasma formation and the timing and mechanism of crossing-over (1974). Kinetic profiles of the synaptinemal complex which plays an important role in the pairing of homologous chromosomes show to accomplish its formation at pachytene and break up itself rapidly at diplotene (MOENS 1968). These evidences lead to suppose that pachytene stage and particularly late pachytene

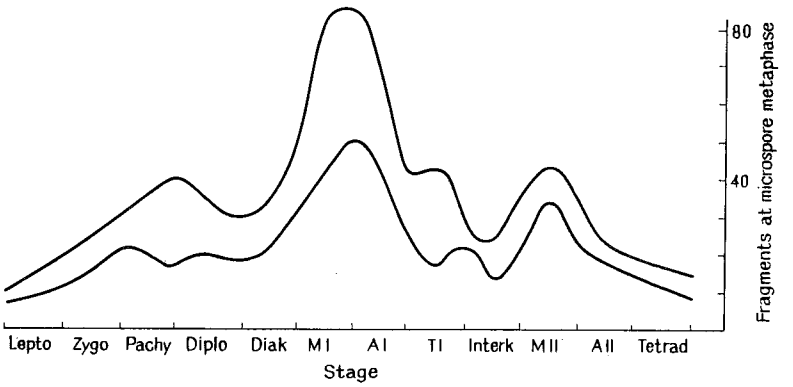


Figure 14. Changes of the radiosensitivity of the chromosome during meiosis. The sensitivity was estimated from the frequency of fragments at the microspore metaphase followig the treatment at different meiotic division stages.

may be the period which biosynthetic and metabolic changes are carried out actively. At the first metaphase chromosomes condense at maximum degree and disperse themselves in the cell and split. The first anaphase chromosomes disjunct and migrate to the poles. The density and movement of nuclear and cytoplasmic substances at pachytene, metaphase and anaphase may have some relation to the radiosensitivity and the modifying effect of nitrogen.

The radiosensitivity of mitosis has been investigated at interphase and thus the spot-light has been directed to the difference of sensitivity between G_1, S and G_2. Concerning the change of the sensitivity during mitotic cycle all three stages have been assumed by one more authors to be the stage of maximum sensitivity. In many reports, however, cells at G_2 and/or mitose (M) are most sensitive. HUS *et al.* (1962) reported that G_2 was more sensitive than G_1 in Chinese hamster cells. The most sensitive period of HeLa cells was reported to be G_2 and M by TERASHIMA and TOLMACH (1963). SCOTT and EVANS (1967) reported that the increase in frequency of two hit type chromosome aberrations was larger in G_2 as compared with that seen at S and G_1. One of barriers to the critical analysis on the sensitive period is the asynchronous division of mitotic cells. The present data in *Trillium* microspore show that chromosomes of G_2 cells are most resistant to X-rays and less influenced by nitrogen.

II. Effect of carbon monoxide on the radiosensitivity of chromosomes of *Trillium* PMCs.

Experiments were carried out in order to investigate the effect of carbon monoxide on the radiosensitivity of microsporocytes. The combination effect of carbon monoxide with nitrogen was also studied.

1. Radiosensitivity of PMCs X-rayed in carbon monoxide at different stages.

Pollen mother cells were irradiated by X-rays at 30 R at the dose rate of 10 R per minute with the operation of 150 KVP, 14 mA and 1.0 mm Cu and 0.5 mm Al filters. Gas treatment was similar to nitrogen treatment with the exception of dark condition. A vacuum desiccator was covered by a dark bag and filled up by pure carbon monoxide at 1 atm pressure. Five or six rhizomes were kept for 16 and 8 hours prior to and after X-ray irradiation respectively. Scoring was done at the first metaphase and anaphase following X-ray irradiation at late zygotene, pachytene, early diplotene and late diplotene-diakinesis. In this experiment plants treated were removed into a room kept at 11 ± 0.5°C to regulate the speed of meiotic division.

Table 13 shows the frequency of aberrations at the first metaphase. The frequencies of simple breaks ranging from 8.8 to 13% were obtained by X-raying in air. The peak of sensitivity seems to be present in early

TABLE 13. Frequencies of aberrations at the first metaphase following X-ray irradiation (30 R) and combined carbon monoxide treatments at different stages of prophase

Treatment	Stage treated	Frequency (%)					Ratio of X-ray to X-ray+CO in iso-chromatid breaks
		Iso-chromatid breaks	Chromatid breaks	Minute acentric fragments	Total simple breaks	Exchanges	
X-ray in air	Late zygotene	7.53	0.52	0.70	8.75	2.09	3.75
	Pachytene	10.05	1.03	0.23	11.31	1.37	3.09
	Early diplotene	10.95	0.87	1.00	12.95	3.11	5.12
	Diakinesis	9.41	1.54	0.52	11.47	3.25	4.34
X-ray in CO	Late zygotene	2.01	0	0	2.01	0.53	
	Pachytene	3.25	0.15	0.15	3.55	0.42	
	Early diplotene	2.14	0.28	0.28	2.72	0.14	
	Diakinesis	2.17	0	0.11	2.28	0	

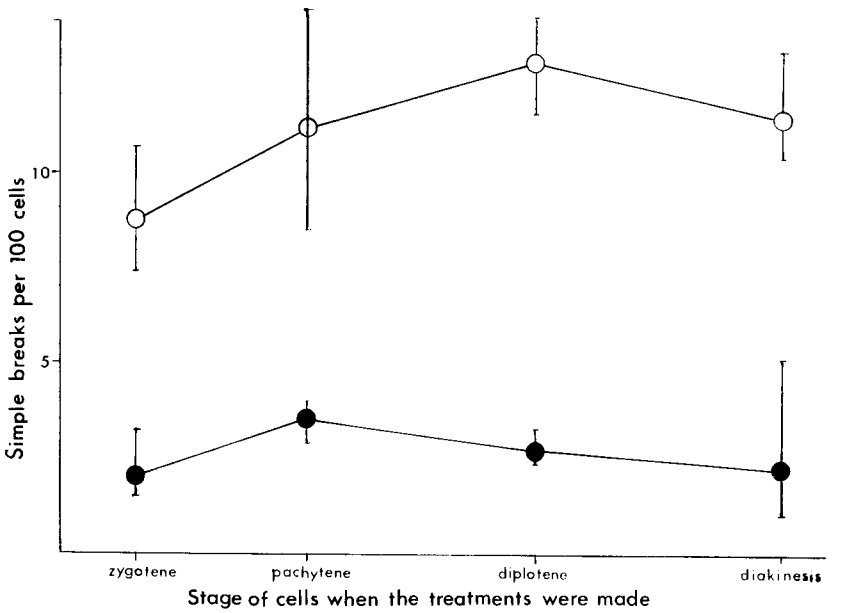


Figure 15. Frequencies of chromasome aberrations at the first metaphase following X-ray irradiation and combined CO treatment at four different stages of prophase. Open circle: X-ray in air. Closed circle: X-ray in CO.

diplotene, though it is not significant. The X-ray irradiation in carbon monoxide led to yield simple breaks with much lower frequencies. The ratio of the frequency in iso-chromatid breaks obtained from X-raying in air to that in CO was maximum, 5.1, at early diplotene and minimum, 3.1, at pachytene. Aberration yields of exchange types were too little to be able to compare with each other. Figure 15 shows the frequency of simple breaks and represents changes of sensitivity during the four stages.

2. Effect of pre- and post-treatment with carbon monoxide.

Effects of pre- and post-treatment of carbon monoxide combined with X-ray treatment were examined. In the pre-treatment group (CO pre) X-rays were given in carbon monoxide and immediately after X-raying carbon monoxide was removed. In the post-treatment group (CO post) the carbon monoxide treatment started after about 12 minutes from X-ray irradiation.

Results summarized in Table 14 show that CO pre-treatment was less effctive. Aberration yield in CO pre-treatment was not different from that in air except in the plants irradiated at diplotene in which the aberration yield was somewhat reduced by carbon monoxide treatment. On the con-

TABLE 14. Frequencies of aberrations at the first metaphase following CO treatment combined with X-ray irradiation at different stages of prophase

Stage treated	Frequency (%) Simple breaks			
	CO pre-treatment	X-ray during CO	CO post-treatment	without CO treatment
Late zygotene	8.33	2.01	4.15	8.55
Pachytene	10.14	3.55	4.23	11.31
Early diplotene	9.65	2.72	3.39	12.95
Diakinesis	—	2.28	3.53	11.47

Stage treated	Frequency (%) Exchanges			
	CO pre-treatment	X-ray during CO	CO post-treatment	without CO treatment
Late zygotene	2.50	0.53	1.12	2.09
Pachytene	0	0.42	0.73	1.37
Early diplotene	1.28	0.14	0.43	3.11
Diakinesis	—	0	0.63	3.25

trary, it seems that the effect of X-ray irradiation in carbon monoxide (throughout CO) which was described in the foregoing section, regardless the stage of treatment, the aberration frequency of CO post-treatment was similar to that of the throughout CO treatment. In Figures 16, 17 and 18 changes of sensitivity are shown. In each treatment frequencies of total aberrations and also of individual aberrant type were less variable throughout four stages.

The aberration freqency of exchange types was often near 0.5% and in two cases these anomalies did not appear. Anyhow, the effect of CO post-treatment was similar to that of raying during CO treatment, and CO pre-treatment was hardly effective.

3. Effects of carbon monoxide observed at microspore division.

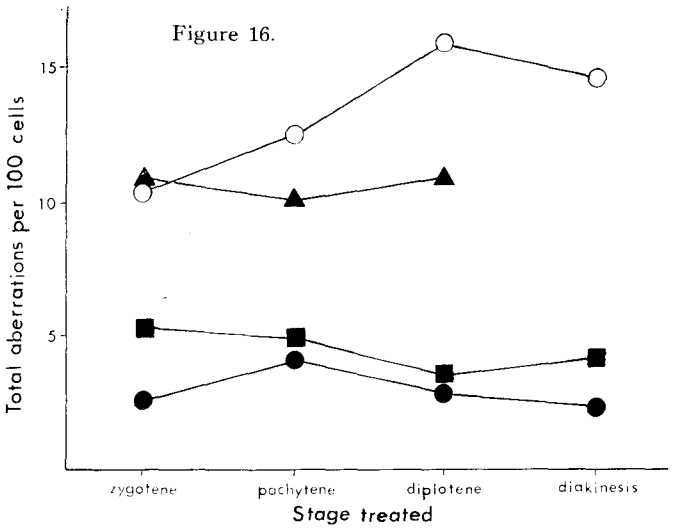
By means of a similar method to that for the observation on nitrogen effect the extent of chromosome fragmentation was estimated at the metaphase of microspore division following X-raying (30 R) in carbon monoxide at different stages of the first meiotic division. In Figure 19 the sum of aberration yields in PMCs rayed at various stages is shown. The frequency of iso-chromatid breaks (B″), chromatid breaks (B′) and minute acentric fragments (mCo) was reduced by carbon monoxide treatment. Frequencies of total fragments were 86.3% in air and 30.3% in CO giving the ratio of 2.84.

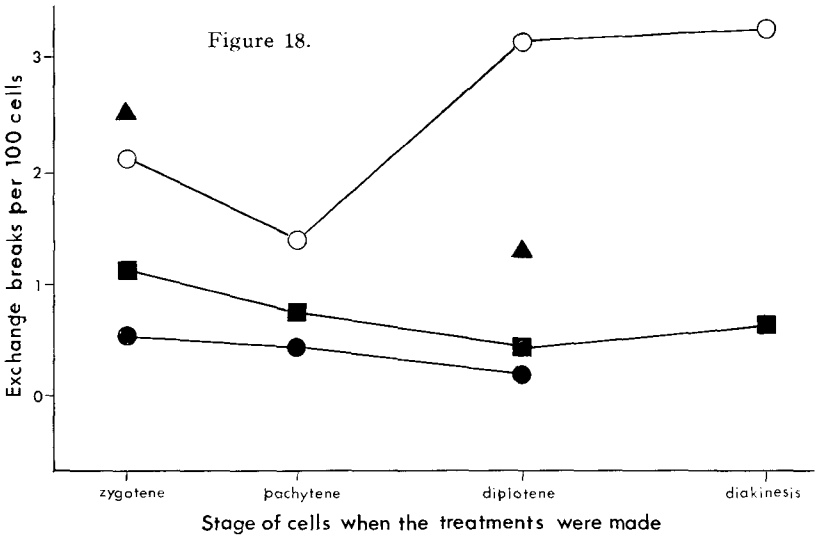
4. Combination effects of carbon monoxide and nitrogen on the sensitivity for X-rays.

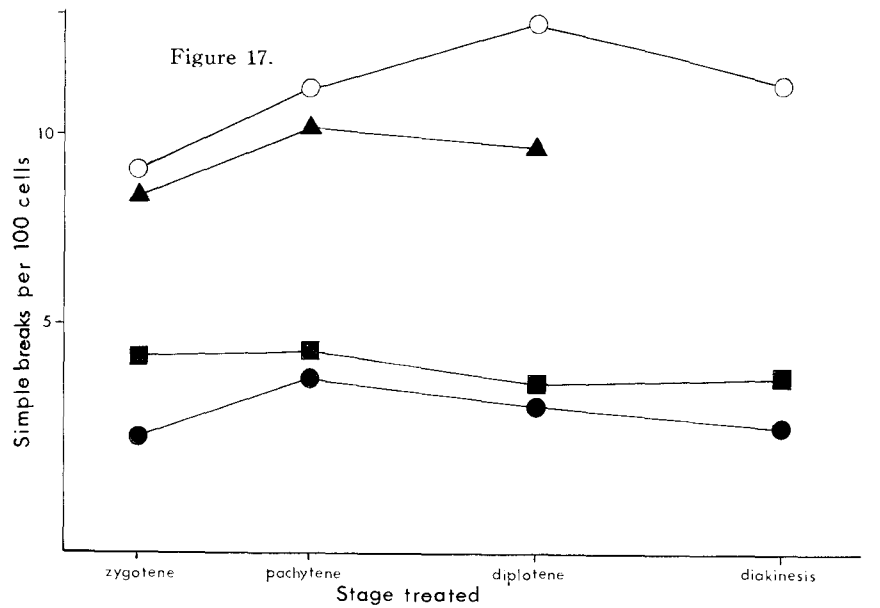
As a preliminary experiment, the treatment prior to as well as after X-ray irradiation in carbon monoxide and nitrogen and these reciprocals were performed. PMCs at diakinesis were rayed with 30 R. The result in Figure 20 suggested that CO post-treatment is more effective in reducing aberration frequency than nitrogen post-treatment.

The programs of experiments were as follows:

1) Plants were kept in nitrogen for 18 hours; 12 hours prior to and 6 hours following X-raying (N_2–X–N_2).
2) The same as above, but in carbon monoxide (CO–X–CO).
3) Twelve hours in carbon monoxide prior to and 6 hours in nitrogen following X-raying. Ten minutes were elapsed before gas exchange to nitrogen after raying (CO–X–N_2).
4) Twelve hours in nitrogen prior to X-raying; 10 minutes were elapsed before exchange to carbon monoxide (N_2–X–CO).







Figures 16, 17 and **18.** Frequencies of chromosome aberrations at the first metaphrse following X-ray irradiation and combined CO treatment at four different stages of prophase. Fig. 16. Total yields of aberrations. Fig. 17. Simple breaks. Fig. 18. Exchange breaks.

-○-: X-ray in air. -▲-: CO pre-treament.
-●-: X-ray during CO. -■-: CO post-treatment.

Some plants were treated by carbon monoxide or nitrogen without irradiation as control groups. Thirty rentogens of X-rays were given at the dose rate of 4.5 R per minute at 17°C. Carbon monoxide treatment was

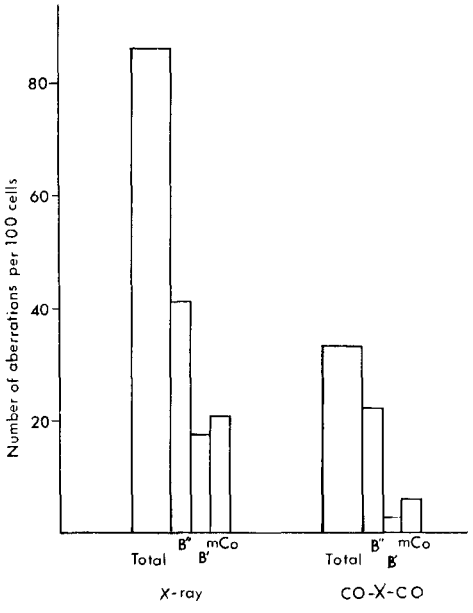


Figure 19. Frequencies of aberrations at the microspore metaphase following X-ray irradiation (50 R) and combined CO treatment at different stages of microsporocyte prophase.

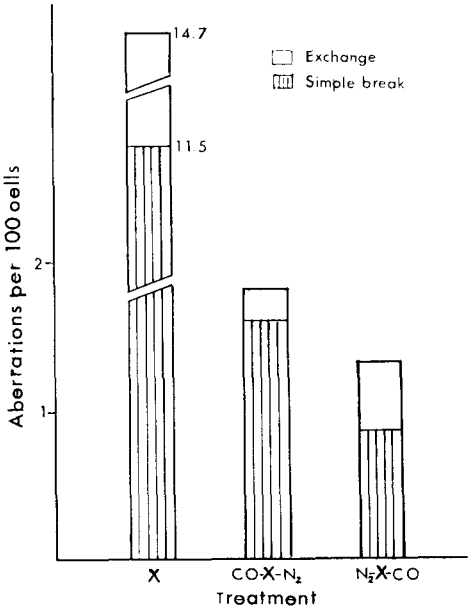


Figure 20. Frequencies of aberrations at the first metaphase induced by treatments of different combinations of X-ray, nitrogen and carbon monoxide at diakinesis.

TABLE 15. Frequencies of the yield of aberrations at the first metaphase by combination treatment of X-ray irradiation with carbon monoxide and nitrogen

Treatment	No. of cells observed	Simple breaks		Exchanges	
		Observed no.	Frequency (%)	Observed no.	Frequency (%)
CO only	1023	1	0.09	0	0
N_2 only	1136	1	0.08	0	0
X in air	1172	110	9.38	36	3.07
N_2–X–N_2	1170	58	4.95	26	2.22
CO–X–CO	1022	42	4.10	8	1.76
CO–X–N_2	719	24	3.35	22	3.07
N_2–X–CO	1260	26	2.06	6	0.47

carried out under dark condition. CO gas used was pure. Scoring of aberrations was done at the first metaphase following the treatment at mid-diplotene.

The data for seven different treatments are given in Table 15 and Figure 21. In the group of CO single or nitrogen single treatment aberrations were scarcely present. The frequency of simple breaks in CO-X-CO treatment was rather lower than that in N_2-X-N_2 treatment. The sum of frequencies of simple breaks and exchange breaks in the CO-X-N_2 treatment was higher than that in the CO-X-CO treatment. Therefore, it is likely that CO-X-CO treatment was more effective in reducing the aberration frequency than CO-X-N_2 treatment. On the whole, it seems that CO post-treatment was more effective in reducing the chromosome aberrations.

BEATTY and BEATTY (1959) suggested that in *Tradescantia* microspores which are X-rayed anaerobic metabolism is utilizable since *Tradescantia* inflorescence is able to live in pure carbon monoxide in the dark for 4 hours without any ill effect. In the present investigation *Trillium* plants were kept in pure carbon monoxide and nitrogen for 24 hours and any physiological changes were not recognizable. It is supposed that living in pure

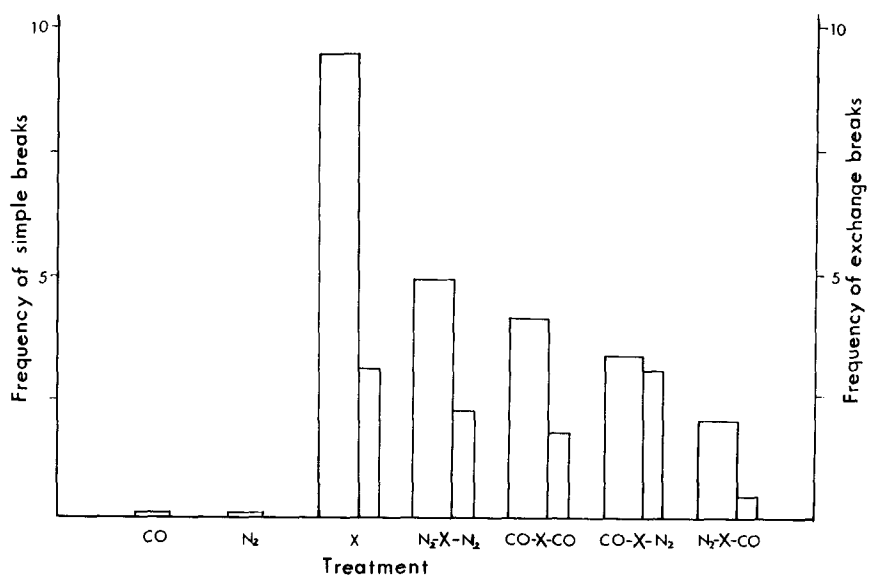


Figure 21. Frequencies of aberrations at the first metaphase by combination treatments of X-ray irradiation, carbon monoxide and nitrogen gas. Broad and narrow columns represent the yields of simple breaks and exchange aberrations, respectively.

carbon monoxide for about 20 hours does not bring about any ill effect, since the development of buds and PMCs is extremely slow in this species. CONGER (1955) pointed out from the data on *Tradescantia* that oxygen exerted its effect by increasing breakage rather than by affecting reunion. NEARY and EVANS (1958) observed that the dose exponents for chromatid breaks, iso-chromatid breaks and chromatid interchanges were same in nitrogen as in air and supported the view that oxygen increases the initial or primary effect of radiation on the chromosomes. WOLFF and LUIPPOLD (1958) discussed that metabolic processes requiring respiratory energy are necessary for the rejoining of breaks from the dose fractionation experiments using metabolic inhibitors including carbon monoxide. They also pointed out there were two types of breaks induced by fractionated X-ray exposure, *i.g.* one fast rejoining process and the other slow rejoining process.

A γ-ray experiment by GILOT-DELHALLE *et al.* (1973) presents to the author an interesting suggestion. In their experiment *Nigella* seeds were irradiated by γ-ray separated by time intervals ranging from 2 to 3 minute with increment of 10 seconds. Fast rejoining processes effective within 2 minutes and 10 seconds were oxygen dependent being suppressed under anoxia and in the treatments of respiratory inhibitors and chelating agents. And all kinds of aberrations *i.e.* breaks, minute and dicentric fragments or rings were involved in this process. Carbon monoxide and/or nitrogen treatment for longer period in the present investigation might affect on the fast reunion process, although from the present data any direct evidence for it can not be obtained.

KIHLMAN (1961) suggested that the main act of carbon monoxide is abolishing of oxygen gradient in cells. If carbon monoxide treatment makes the same condition as in the case of nitrogen treatment for chromosome breakage the present results can be simply interpreted as that X-ray irradiation was done under anoxia which was induced by carbon monoxide. However, the different extents of aberration yield between various combination treatments suggest the existence of a characteristic effect of carbon monoxide.

It has become clear by several works that the effect of oxygen is an immediate one and oxygen must be present during X-ray irradiation in order to produce an effect (GILES and RILEY 1950, HOWARD-FLANDERS and MOORE 1958). The irradiation of water produces H and OH radicals and H radicals in the presence of air interact with oxygen to give rise to HO_2 and H_2O_2. The investigation of HOWARD-FRANDERS and MOORE (1958) showed that the oxygen supplied immediately (5–10 seconds) after X-ray irradiation in

nitrogen had no influence to enhance a radiation damage in bacteria. In the present experiment, however, it should be considered that *Trillium* PMCs were kept under fully anaerobic condition by nitrogen treatment for 20 hours.

WOLFF and LUIPPOLD (1959) postulated that the irradiation independently affects the number of chromosome breaks in the cell and the time during which they will remain open. The presence of oxygen at the time of irradiation incerases the amount of damage. This is reflected both in an increased number of breaks and in allowing breaks to remain open longer. If the respiration is inhibited, less amount of ATP is produced and breaks can not rejoin. It is likely that the combination treatments of carbon monoxide and nitrogen act at both the time of the irradiation and the time after irradiation.

It is necessary to discuss on the time elapsed in nitrogen and/or carbon monoxide. About one day under gas treatments is 1/180 of whole duration of meiosis in PMCs of *Trillium*. This corresponds to 4 hours in *Lilium* which takes about one month for the accomplishment of meiosis. The works by VAN'T HOF (1965) showed that the mitotic cycle duration was 8.8 hours in *Impatiens balsamina*, 17.4 hours in *Allium cepa*, 20.0 hours in *Tradescantia paludosa* and 10.75 hours in *Crepis capillaris*. The same author showed in *Pisum sativum* root the total cycle was 17.9 hours (VAN'T HOF 1968). Mitotic duration in *Vicia faba* is 19.5 hours at 19°C including 2.0 hours duration from prophase to telophase and perhaps 1.4 hours of prophase (EVANS and SCOTT 1964). Average mitotic cycle may be ranging from 15 to 30 hours in many higher plants. Tritium labelling experiments in *Trillium grandiflorum* gave 120 hours as total cycle time of mitosis (GRANT 1965).

When one will consider the mitotic cycle in the same points of view of the microsporogenetic cycle, 20 hours duration under gas treatment in 6 months of meiotic cycle of *Trillium* is equivalent to 5.4 minutes in the mitotic cycle of *Vicia faba* root-tip cells and 33 minutes in that of *Trillium* root cells. Thus, it is considered that 20 hours gas treatment is not so excess to bring about appreciable physiological damages.

Acknowledgment

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Summary

1) The present paper dealt with the change of radiosensitivity of chromosomes during meiosis in PMCs of *Trillium kamtschaticum* PALL. and the modifying effect of nitrogen and carbon monoxide on the sensitivity. Chromosome aberrations induced by X-ray irradiation were used as a measure of the sensitivity.

2) PMCs at zygotene, pachytene and diplotene were irradiated with X-rays (30 R) and the radiosensitivity was estimated by scoring induced isochromatid breaks, chromatid breaks, minute acentric fragments and exchange type aberrations at the first metaphase. Cells at pachytene showed relatively high sensitivity for X-rays. The nitrogen treatment largely reduced aberration yield at all three stages tested without delay of the division process. The reducing effect of nitrogen and carbon monoxide was highest at late pachytene.

3) For the estimation of radiosensitivity of chromosomes at different stages during meiosis, it was required to set an index showing the relative sensitivity. Some experiments were performed to choose a reliable standard out of micronuclei at tetrad, dwarf pollen and fragments at metaphase of pollen mitosis. As the result, it was found that the frequency of fragments appearing at the microspore metaphase can be used as the standard of radiosensitivity of chromosomes during meiotic cycle.

4) Plotting of the sensitivity during meiotic cycle showed a roughly bimodal curve having one major peak at the first metaphase and a minor peak at the second metaphase. The sensitivity of prophase cells showed an irregular curve dropping down at mid-pachytene and having a small peak at late-pachytene.

5) The chromosome aberrations, produced by X-raying at the first metaphase and observed at the microspore metaphase, was reduced about to half by the nitrogen gas treatment prior to and after irradiation. Nitrogen was also effective at pachytene. Carbon monoxide also affected to reduce chromosome aberrations by X-rays. Treatments of X-ray, nitrogen gas and carbon monoxide in various combinations reduced frequency of aberrations than X-ray irradiation only in nigtrogen or carbon monoxide.

6) As to the effects of nitrogen and carbon monoxide it is considered that the reduced oxygen in cells by gas exchange affects both the occurrence of chromosome breaks by X-rays and the rejoining system. It is also suspected that the inhibition of respiration by nitrogen and carbon monoxide prevents the movement of chromosomes and the rejoining of breaks so that break ends can not form exchange type aberrations but remain being capable of restitution.

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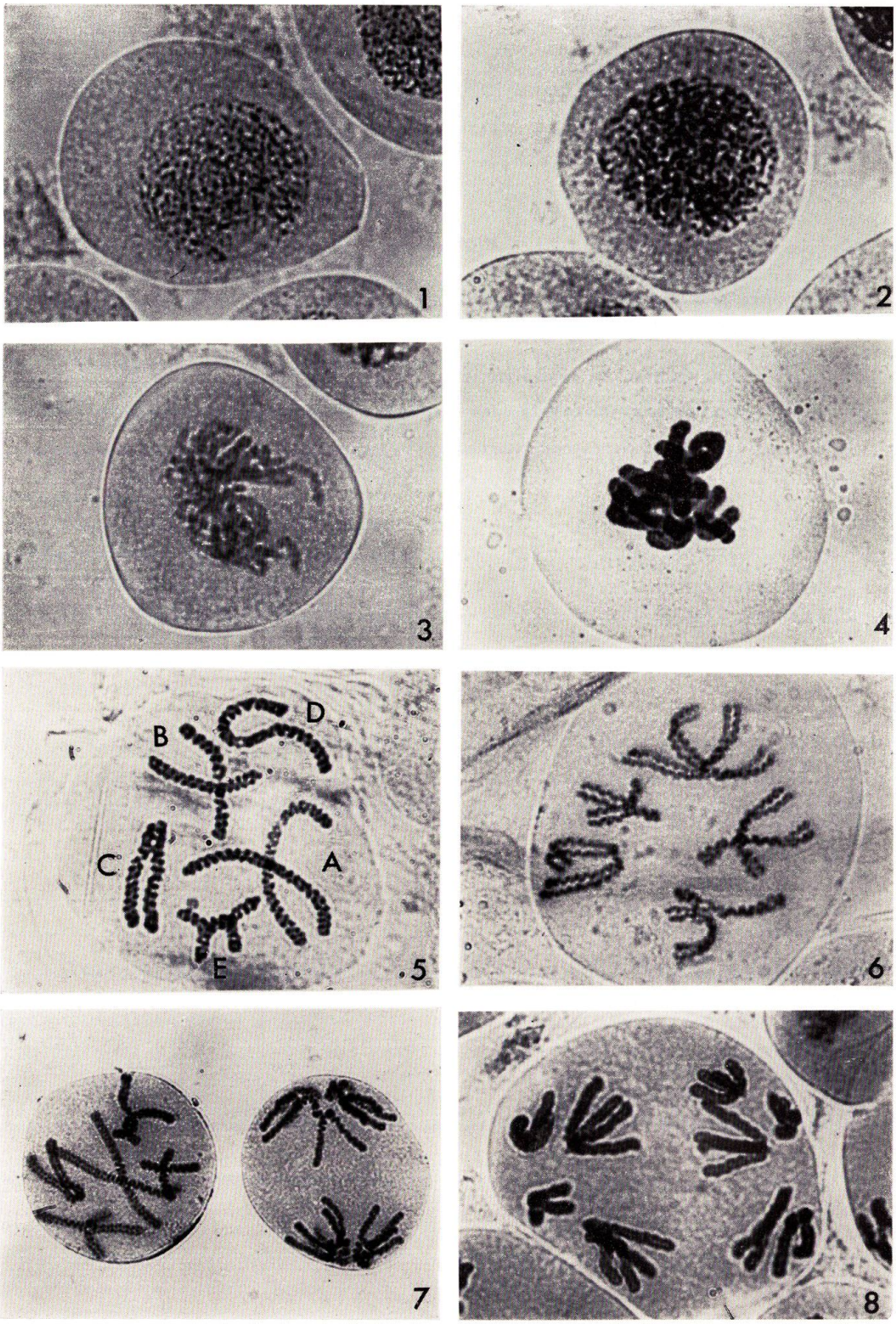
Plate I

Figs. 1-4. Prophase cells at the time of treatments.
1. Zygotene cell. 2. Pachytene cell.
3. Late diplotene cell. 4. Diakinesis cell.

Figs. 5-6. A complete set of chromosomes at the first metaphase, viz. A-, B-, C-, D- and E-chromosomes.

Fig. 7. Cells at the first metaphase and anaphase.

Fig. 8. Normal cell at the first anaphase.



Hajime Kanazawa: X-ray sensitivity of meiotic chromosomes

Plate II

Figs. 1–8. Normal cells at various stages.

1–2. Cells at interkinesis.

3–4. Cells at the second metaphase.

5–6. Cells at the early second anaphase.

7. Cells at the second telophase.

8. A tetrad cell.

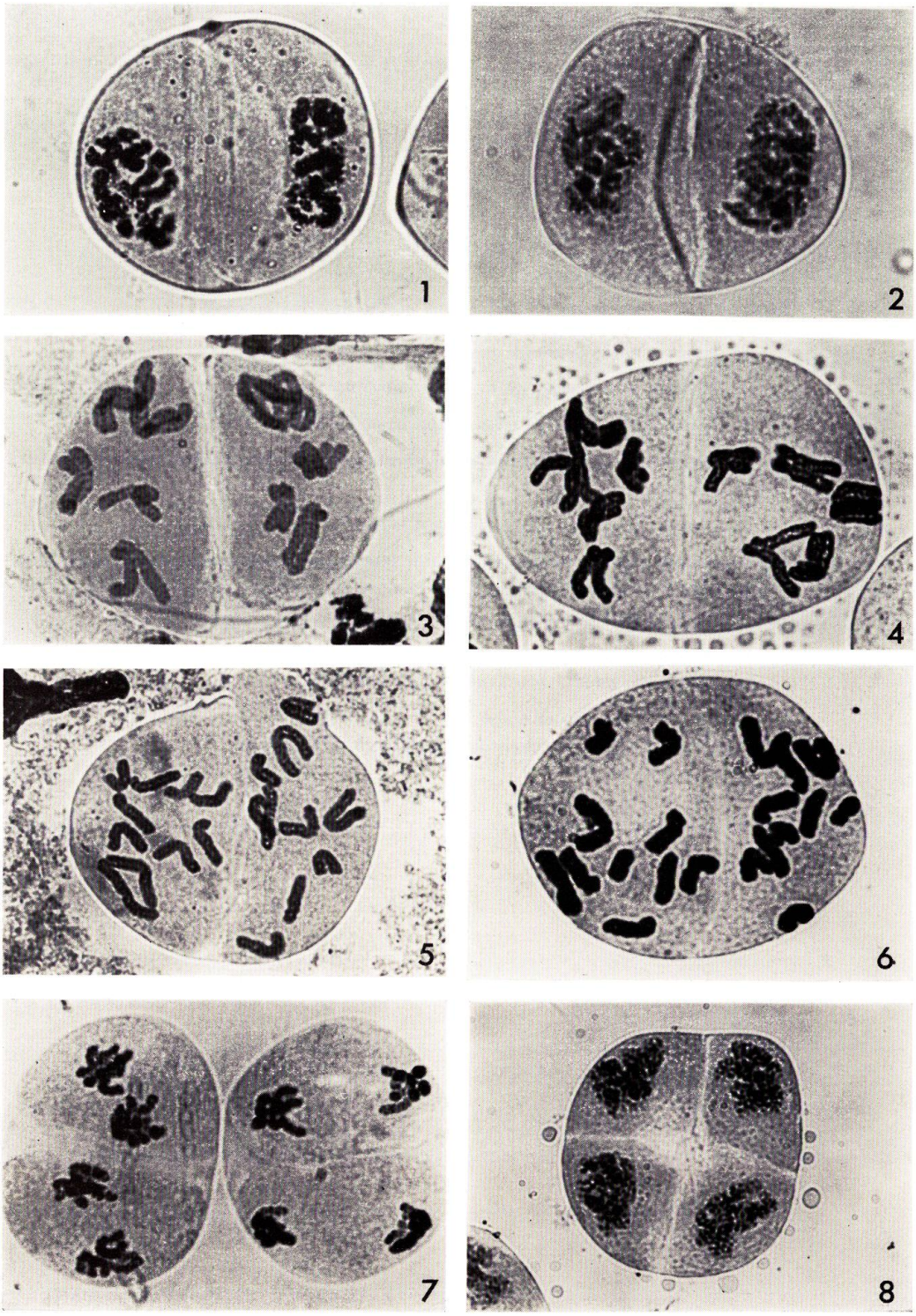


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Plate III

Figs. 1–8. Various types of aberrations at the first metaphase.

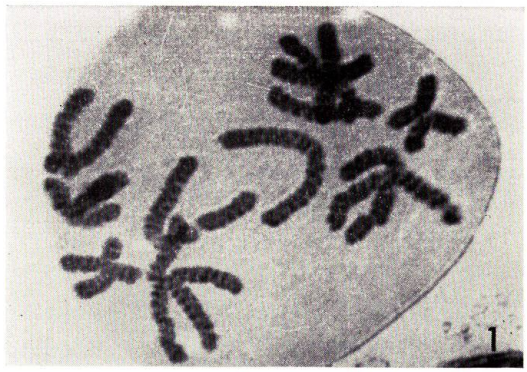
1. Iso-chromatid breakage in C-chromosome.
2. Iso-chromatid breakage in D-chromosome.
3. Small fragment released perhaps from D-chromosome.
4. Iso-chromatid breakage in C-chromosome and chromatid breakage in A-chromosome.
5. Chromatid-chromatid interchange between C- and B-chromosome.
6. Intrabivalent chromatid-chromatid interchange in D-bivalent and two chromatid-chromatid interchanges between E- and B-chromosome and between A and C-chromosome.
7. Intrabivalent chromatid-chromatid interchanges in C-chromosome and iso-chromatid breakage in the same arm.
8. Chromosome-chromosome interchange between A and D-chromosome and chromatid-chromatid interchange between C and A-chromosome.

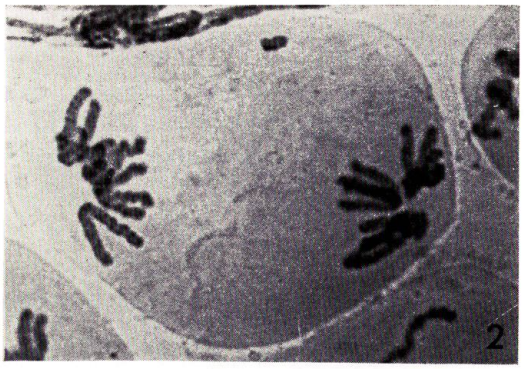
Hajime Kanazawa: X-ray sensitivity of meiotic chromosomes

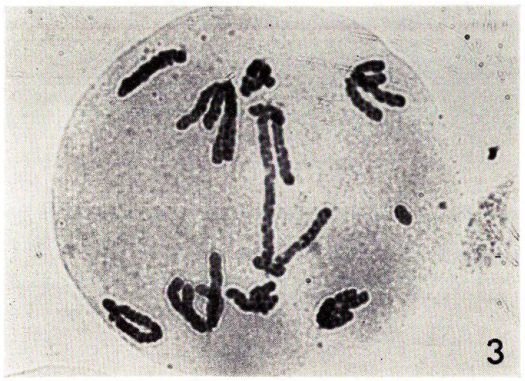
Plate IV

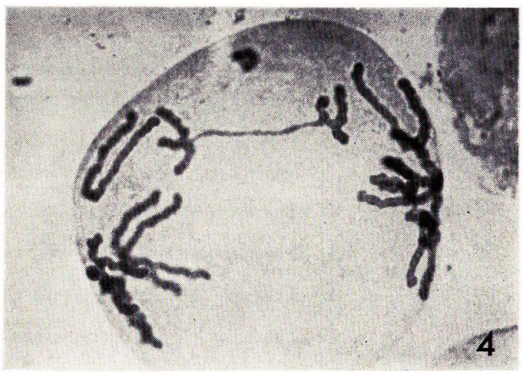
Figs. 1–8. Various types of aberrations at the first anaphase.

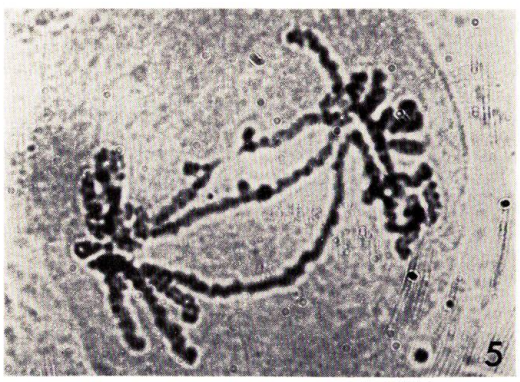
1. Chromatid fragment in D-chromosome.
2. Anaphase cell with a fragment.
3. Bridge configulation of C-chromosome accompanied with a fragment.
4. Bridge configulation of E-chromosome accompanied with a fragment.
5. Two two-side arm bridges and a bridge without fragment.
6. Bridge without fragment.
7. Two-side-arm bridges in D-chromosome and E-chromosome.
8. Lagging chromosome at the first telophase.

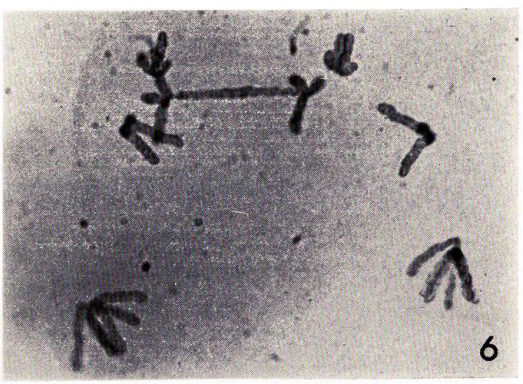


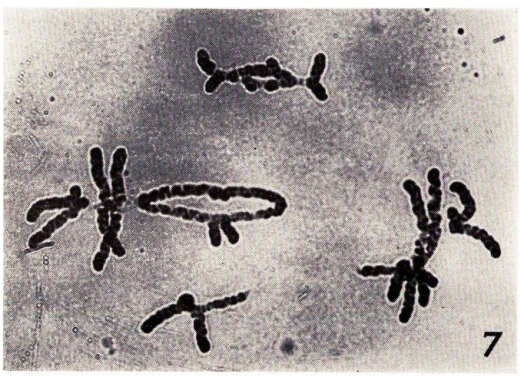


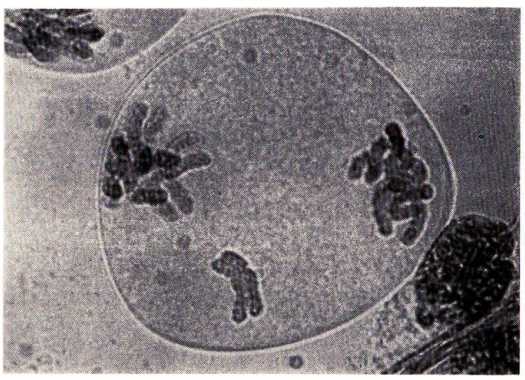










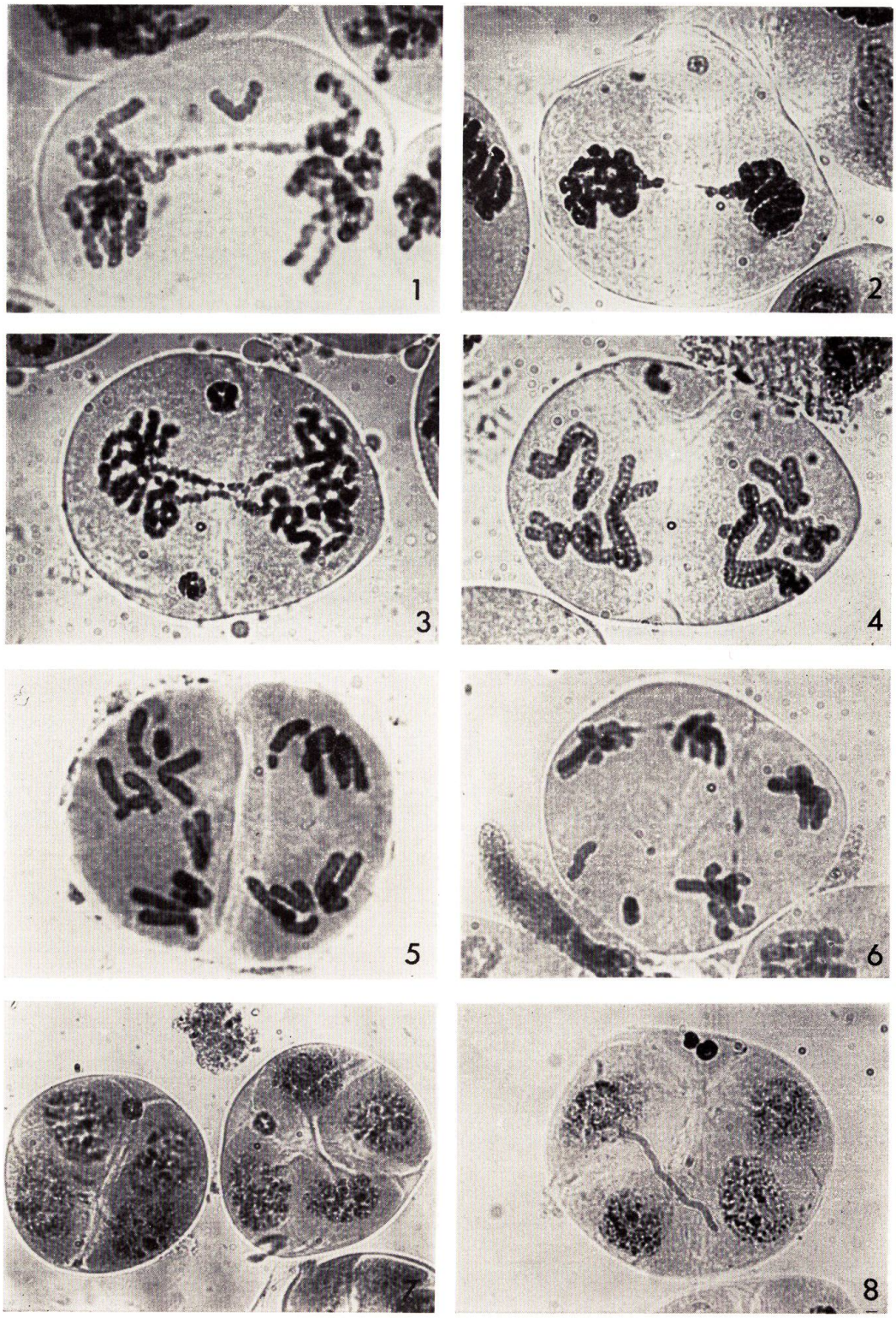


Hajime Kanazawa: X-ray sensitivity of meiotic chromosomes

Plate V

Figs. 1–8. Fragmentation of chromosomes and formation of micronuclei.

1. Bridge accompanied with a fragment at the first anaphase.
2. Ruptured bridge and fragment developing into a micronucleus at the first telophase.
3. Two bridges crossing through septum and two micronuclei-like fragments at later interkinesis.
4. The second metaphase cell with a dwarf daughter cell.
5. The second anaphase cell containing a chromosome with a gap.
6. The second anaphase cell with two fragments and two bridges.
7. Tetrad with a micronucleus or a minute cell.
8. Tetrad with two micronuclei.

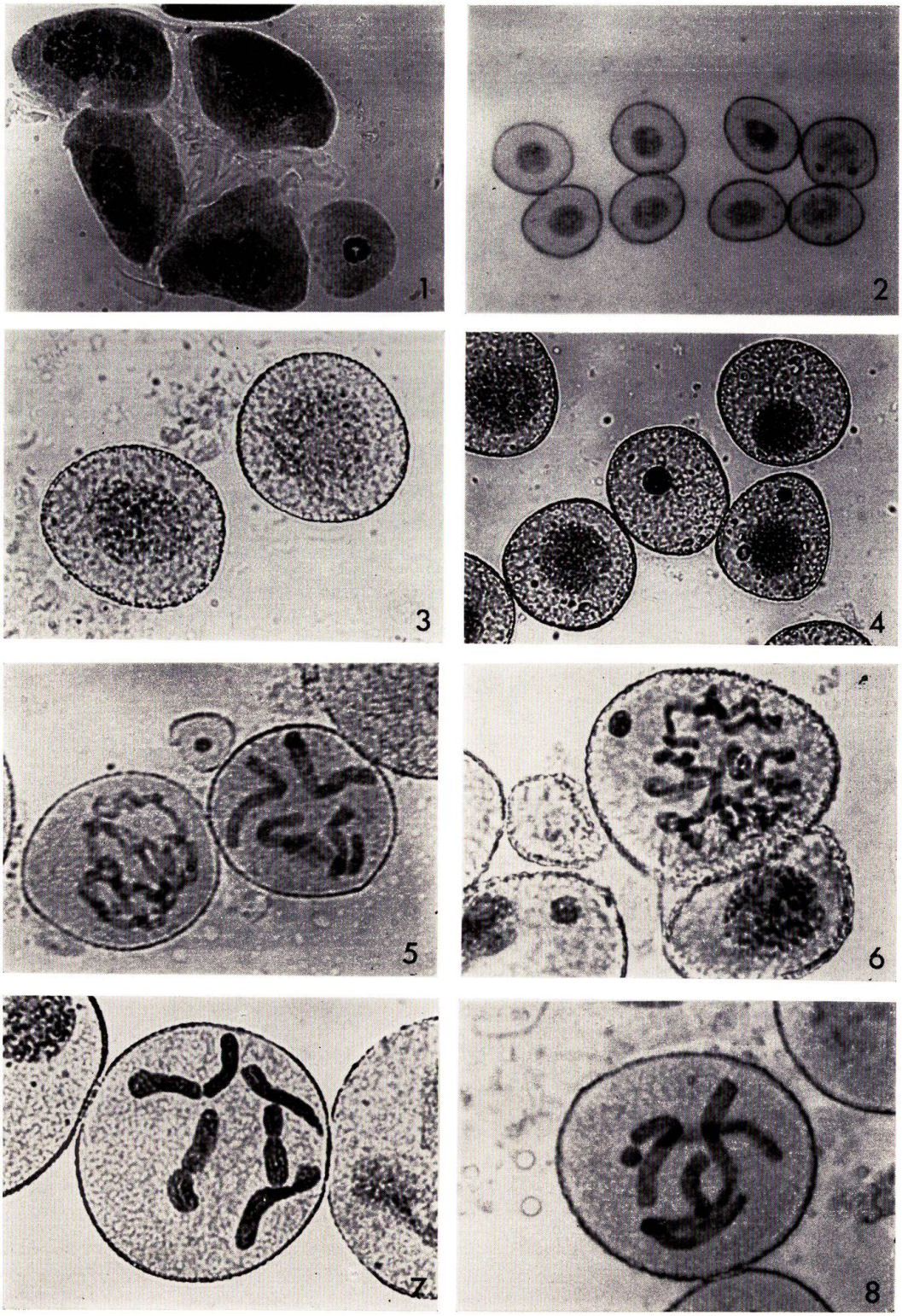


Hajime Kanazawa: *X-ray sensitivity of meiotic chromosomes*

Plate VI

Figs. 1–8. Cells at various stages of microspore division.

1. Dispersing tetrad. A small cell is assumed to develop into dwarf pollen.
2. Cells at early G_1 period. One cell has two micronuclei.
3. Normal pollen at interphase.
4. Pollen bearing one micronucleus co-existing with a major nucleus.
5. Normal pollen at prophase and metaphase, and a dwarf pollen.
6. Prophase cell with a micronucleus.
7. A complete set of chromosomes at metaphase.
8. Metaphase cell with a fragment.



Hajime Kanazawa: *X-ray sensitivity of meiotic chromosomes*

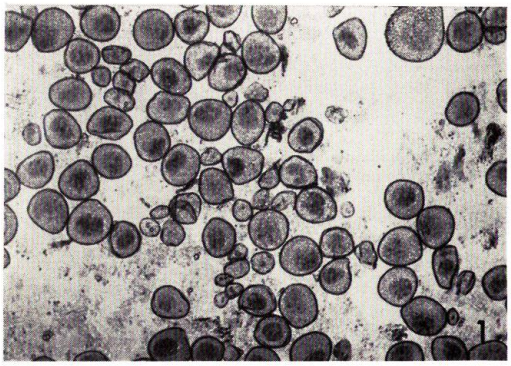
Plate VII

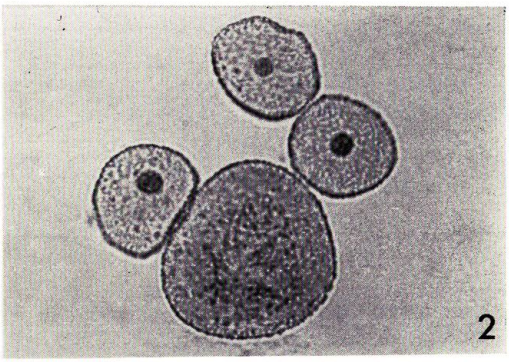
Figs. 1–8. Dwarf pollen and polyploid cells appeared at the microspore division following treatments at the meiotic prophase.

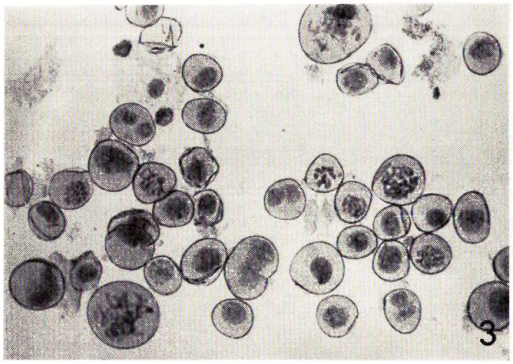
1. Interphase cells. Many dwarf pollen are present.
2. Three dwarf pollen and a normal pollen at late interphase.

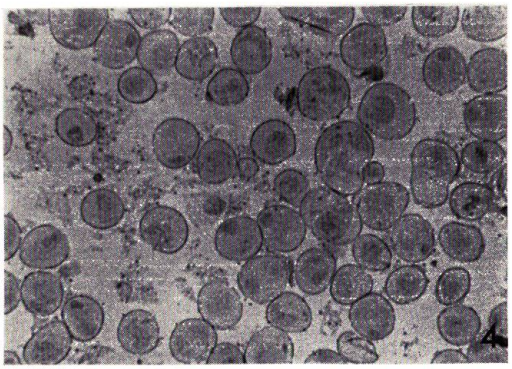
3–4. Polyploid pollen at prophase with round or irregular forms.

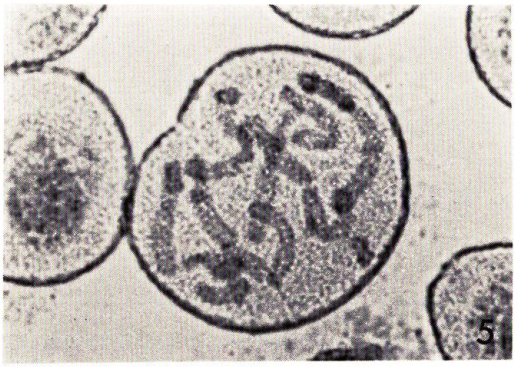
5. Triploid metaphase cell with fragments.
6. Metaphase cell with two fragments.
7. Polyploid cell with numerous fragments.
8. Abnormal pollen with deficiencies in three chromosomes.

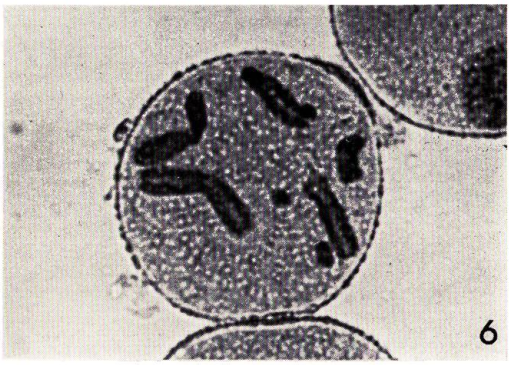


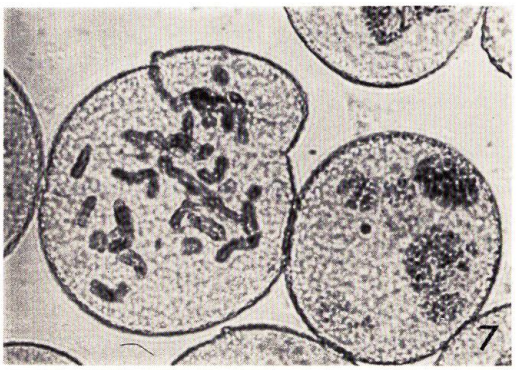


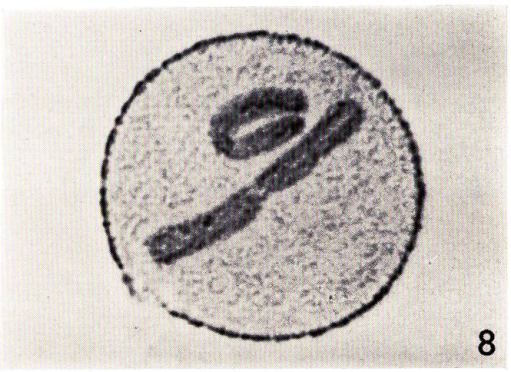












Hajime Kanazawa: X-ray sensitivity of meiotic chromosomes