X-RAY SENSITIVITY OF METAPHASE CHROMOSOMES IN CULTURED POLLEN TUBES OF TRADESCANTIA

by

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RINGKASAN

Tepung sari *Tradescantia paludosa* Anderson & Woodson dikecambahkan pada gelas-gelas obyek yang telah dilapisi dengan medium lactose agar. Untuk setiap percobaan digunakan 4 kelompok yang masing-masing terdiri atas 8 gelas obyek. Tiga kelompok, masing-masing gelas obyek diletakkan mendatar di dalam kotak plastik dengan pengaturan sedemikian rupa sehingga bagian-bagian yang mengandung kecambah-kecambah tepung sari berada tepat pada daerah-sasaran-penyinaran (kira-kira seluas 100 cm^2) sinar-X. Dalam kotak plastik telah ditempatkan kertas Kleenex yang lembab dengan maksud untuk memberikan keadaan pertumbuhan yang optimum bagi perkecambahan tepung sari. Kelompok pertama, kedua dan ketiga masing-masing mendapatkan dosis penyinaran total 310 r (155 r/menit)- ialah penyinaran terhadap chromosoma-chromosoma interphase (semenit sesudah penaburan tepung sari), chromosoma-chromosoma prophase (8 jam sesudah penaburan) dan chromosoma-chromosoma metaphase (18 jam sesudah penaburan dan semenit sebelum fiksasi). Kelompok keempat, sebagai kontrol, tak mendapatkan penyinaran. Alat sinar-X (Standard X-ray Co., Model E) digunakan pada kekuatan 80 kv, 5 ma, dengan filter aluminium setebal 1.2 mm, sedang jarak penyinaran 13 cm. Tepung sari, sebelum maupun sesudah penyinaran, dikecambahkan di dalam tabung perkecambahan yang dibungkus plastik dan dimasukkan dalam incubator dengan suhu 23 ± 2°C.

Hasil dari tiga percobaan ulangan menunjukkan bahwa chromosoma-chromosoma metaphase mempunyai kepekaan terendah terhadap penyinaran (8.8 patahan per 100 sel) bila dibandingkan terhadap chromosoma-chromosoma prophase (33.4 patahan per 100 sel) dan chromosoma-chromosoma interphase (139.7 patahan per 100 sel) di dalam siklus mitosis yang bersamaan; sedangkan kelompok kontrol hanya memberikan 4.2 patahan per 100 sel.

Kepekaan yang rendah terhadap sinar-X bagi chromosoma-chromosoma metaphase tersebut diikuti juga dengan tak adanya penukaran-chromatida. Kenyataan ini mungkin sebagai akibat terlalu jauhnya jarak antara potongan-potongan chromosoma yang tak sejenis dan ketidak mampuan chromatida-chromatida yang bersangkutan untuk membentuk rekombinasi. Piknosis pada chromosoma-chromosoma hanya tampak pada perlakuan kelompok prophase, sedangkan pada perlakuan kelompok-kelompok interphase dan metaphase tak menunjukkan gejala tersebut.

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SUMMARY

Pollen tubes of *Tradescantia paludosa* Anderson and Woodson Sax's clone-3 were cultured on lactose-agar medium coated on the microslides. From the irradiated freshly sown pollen and pollen tubes, various types of chromatid aberrations were observed : chromatid and isochromatid aberrations, chromatid exchanges, and the rare occurrence of chromatid rings and centromere breaks.

Gaps were found in all irradiated stages but they were not included as chromatid aberrations. Result of there repeated experiments indicate that metaphase chromosomes have the lowest radiosensitivity (8.8 breaks per 100 cells) when compared to prophase (33.4 breaks per 100 cells) and interphase chromosomes (139.7 breaks per 100 cells) of the same mitotic cycle. The low radiosensitivity of metaphase chromosomes was accompained by absence of chromatid exchange in the metaphase treated groups. The lack of chromatid exchange indicates, presumably the changes in spatial relationship among the broken ends of the non-homologous chromatids and inability of these chromatids to form recombinants. Pycnosis, or stickness of chromosomes, was observed in considerable numbers of cells irradiated at prophase stage but not in those chromosomes treated at interphase and metaphase stages.

I. INTRODUCTION AND LITERATURE REVIEW

Early in this century two French physicians, Bergonie and Tribondeau, investigated the biological effects of the newly discovered X-rays. They found that tissues containing cells that were relatively immature or cells that were in an active state of division were more sensitive to radiation than were other tissues. They proposed an hypothesis that all cells of relatively undifferentiated tissue which are actively dividing are sensitive to radiation. This was known as the "law of Bergonie and Tribondeau".

Following this discovery, many investigators have studied radiosensitivity of cells in different stages of mitosis and meiosis in a wide range of plant and animal species. They attempted to describe the effects of X-rays, ultraviolet rays and other types of radiation on cells and to identify the most sensitive stage of the cell cycle to radiation. The definition of sensitive stage varied. Such criteria as cell-death or survival value, delay or retardation of cell division, the production of aberrations or fragmentation of the chromosomes, changes in metabolism, and other cytochemical changes, have been used to judge the effects of radiation.

In the last 40 years, considerable attention has been given to the changing sensitivity of cells to X-rays during the course of cell division, especially as evidenced by chromosomal damage or chromosomal aberrations. Although almost every stage has been claimed as the most sensitive part of the cell cycle by different investigators, the majority of the investigators agreed that the metaphase stage was the most sensitive one. These differences in opinion could be ascribed, in some cases, to differences in experimental organisms used or the type of experimental methods applied, as well as additional modifying factors present during the course of experimentation.

Kraus and Ziegler (1906) found evidence that cells were most sensitive to X-rays at the time of organization of the equatorial plate. From the studies on chick tissue culture, *Chorthippus*, the mouse lymphoma chromosomes, it was shown that interphase or very early prophase was a stage of high sensitivity (Sparrow, 1951). Koller (1946) showed in his study of pollen mitotic chromosomes of *Tradescantia* that the highest sensitivity is reached at the end of interphase, when the chromosomes divide longitudinally into two sister chromatids. Prophase has been reported to be the most sensitive stage by a number of investigators. Sax (1940), Sax and Swanson (1941), Darlington and La Cour (1945), Delone, Egorov and Antipov (1966) used *Tradescantia* microspores, *T. paludosa* Anderson and Woodson, for studying the sensitivity change through the mitotic cycle. The earlier studies of Sax (1940) demonstrated prophase to be the most sensitive stage. Later, Sax and Swanson (1941) found that maximum sensitivity occurred just before mid-prophase and then decreased as metaphase was approached. Darlington and La Cour (1945) and Delone, Egorov and Antipov (1966) reported similar results. Bishop (1950) using the same material but applying the so-called "double scoring method" as described by Sparrow (1951), found metaphase to be most sensitive stage. Singh (1963), after irradiation of premeiotic inflorescence of tomato, reported highest sensitivity in prophase I with a second sensitivity peak in metaphase II.

The studies of many investigators have found metaphase to be the most sensitive stage of the cell cycle. Whiting (1945) indicated in her studies on unlaid eggs of waap, that metaphase I of meiosis was most sensitive to irradiation. Hatchability, interpreted as dominant lethals caused by fragment loss, was used to determine the change in sensitivity during the meiotic cycle. Sparrow (1951) from his studies on irradiated flower buds of *Trillium erectum* L. showed metaphase I to be a stage of high sensitivity. Conger (1947) found metaphase to be approximately 37 times as sensitive as interphase in onion root-tip cells. Similar results have been reported by Davidson (1958) in his studies on *Vicia faba* L. roots. Sidorov and Sokolov (1966) in their studies on *Crepis capillaris* (L) Wallr. showed high radiosensitivity of the anaphase, metaphase and late prophase chromosomes, and a gradual decrease of sensitivity in early prophase, G_2 (period between DNA-synthesis and prophase) and especially G_1 (period between telophase of the preceding cell cycle and DNA-synthesis). Murakami

(1967; 1969) reported that metaphase and telophase were sensitive stages during the mitotic cycle of silkworm. Similar results have been reported by Zermeno and Cole (1969) in Chinese hamster cells.

Ballardin and Metalli (1963) have used *Artemia*, an organism in which the timing of meiosis is facilitated by synchronous development of the oocytes, each stage having its characteristic position within the genital tract. The criterion of X-ray damage was hatchability inhibition, interpreted as dominant lethality, a criterion established by Whiting (1945) in her experiments on *Habrobracon*. They found that hatchability was lowest when eggs were irradiated at the earliest prophase stage and increased throughout prophase to a maximum at metaphase. This is in direct contrast to the data in *Harbrobracon*.

From the available references, it was shown that the majority of the former investigators found metaphase to be the most sensitive stage to radiation. Those results have been obtained by analyzing chromosomal aberrations at metaphase of the second post-treatment division cycle. However, very few studies have been done concerning the "direct" hits of radiation on the metaphase chromosomes. This lack of information was due either to the relative briefness of this stage or to the complications resulting from physiological effects. Pycnosis, or stickiness of metaphase chromosomes occurred immediately after irradiation in most of the plant materials used.

The pollen grains of *Tradescantia* have six large chromosomes and the mitotic stages of the developing grains are more or less synchronized. The technique of culturing pollen tubes in artificial medium makes it possible to obtain a monolayered distribution of grains and also provides planar distribution of chromosomes to the slide. This system, then, provides a suitable material for observations of radiation effects on chromosomes.

The present study deals with breakage of metaphase chromosomes in cultured pollen tubes induced by X-irradiation. Metaphase chromosomes can be readily studied since they to do not become pycnotic immediately after irradiation as shown in other cytological materials. Colchicine was used to accumulate a large number of metaphase chromosomes for observation.

II. MATERIAL AND METHODS

Pollen of *Tradescantia paludosa* Anderson and Woodson, Sax's clone-3, was used in these experiments which were conducted during the summer season of 1970. The pollen was collected at 11 : 00 a.m. from the full-blown flowers of the greenhouse-grown plants. Pollen from several flowers was mixed and placed in a desiccator containing calcium chloride for four hours in the dark before sowing. It was germinated on slides coated with a film of 12 per cent lactose and 1.5 per cent agar medium, supplemented with 0.04 per cent colchicine in order to secure a large number of metaphase figures in the generative nuclei of the pollen tubes.

Sowing the pollen into the culture medium was done by means of a camel's hair brush. Standard staining dishes containing two pieces of Kleenex (free from chemicals and perfume) moistened with five milli-liters of distilled water were used as a germinating chamber. This provided an environment in the chamber nearly saturated with moisture. Immediately after sowing, the slides were placed in the germinating chamber, wrapped with Saran wrap, and allowed to germinate and grow in the incubator at 23 ± 2°C for 18 hours.

X-rays were generated from a model-E X-ray unit (Standard X-ray Co.), operating at 80 kv and 5 ma with a 1,2 mm aluminum filter.

Pollen was irradiated 13 cm from the filament of the X-ray tube, at which distance a dose rate of 155 r per minute and an exposure field with a diameter of about 10 cm was obtained. The dosage was determined with a Victoreen Condenser R-meter at 24 ± 1°C at a barometeric pressure of 752 ± 10 mm Hg. A total dose of 310 r was administered by exposing slides to X-irradiation for two minutes in a plexiglass chamber (treatment chamber) as shown in Figure I. The treatment chamber contained two pieces of Kleenex moistened with eight milliliters of distilled water and the chamber was wrapped with Saran wrap at the time of treatment. The slides were placed in two rows of four slides each lying side by side, and were so arranged that culture-bearing portions of these slides would coincide with the radiation field.

Four groups of eight slides were used in each experiment. One group represented the control group and the three other groups were treated at three different stages of the mitotic cycle.

The four groups referred to in this study are : Group A - established by irradiating freshly sown pollen, and then returning them to the germination chamber to continue the 18 hour growth period. Group B - irradiated after the pollen had been sown for eight hours; after irradiation the same procedure as in Group A was followed. Group C - irradiated at the end of 18 hours of growth. Group D - control without irradiation.

All the slides of the four different groups were fixed in Gates' fixative immediately after Group C had been irradiated at the end of the 18 hour growth period. Irradiated interphase (0 hours after sowing or freshly sown pollen) and prophase (8 hours after sowing) chromosomes were fixed 18 hours and 10 hours after irradiation, while irradiated metaphase chromosomes were fixed 1.5 minutes after irradiation. The control groups were fixed 18 hours after sowing. The slides were stained and the permanent preparations were made according to the tehnique described by Ma (1967). An outline of the technique is shown in Appendix I. Three replications (one per week) of each experiment were made following the same procedures.

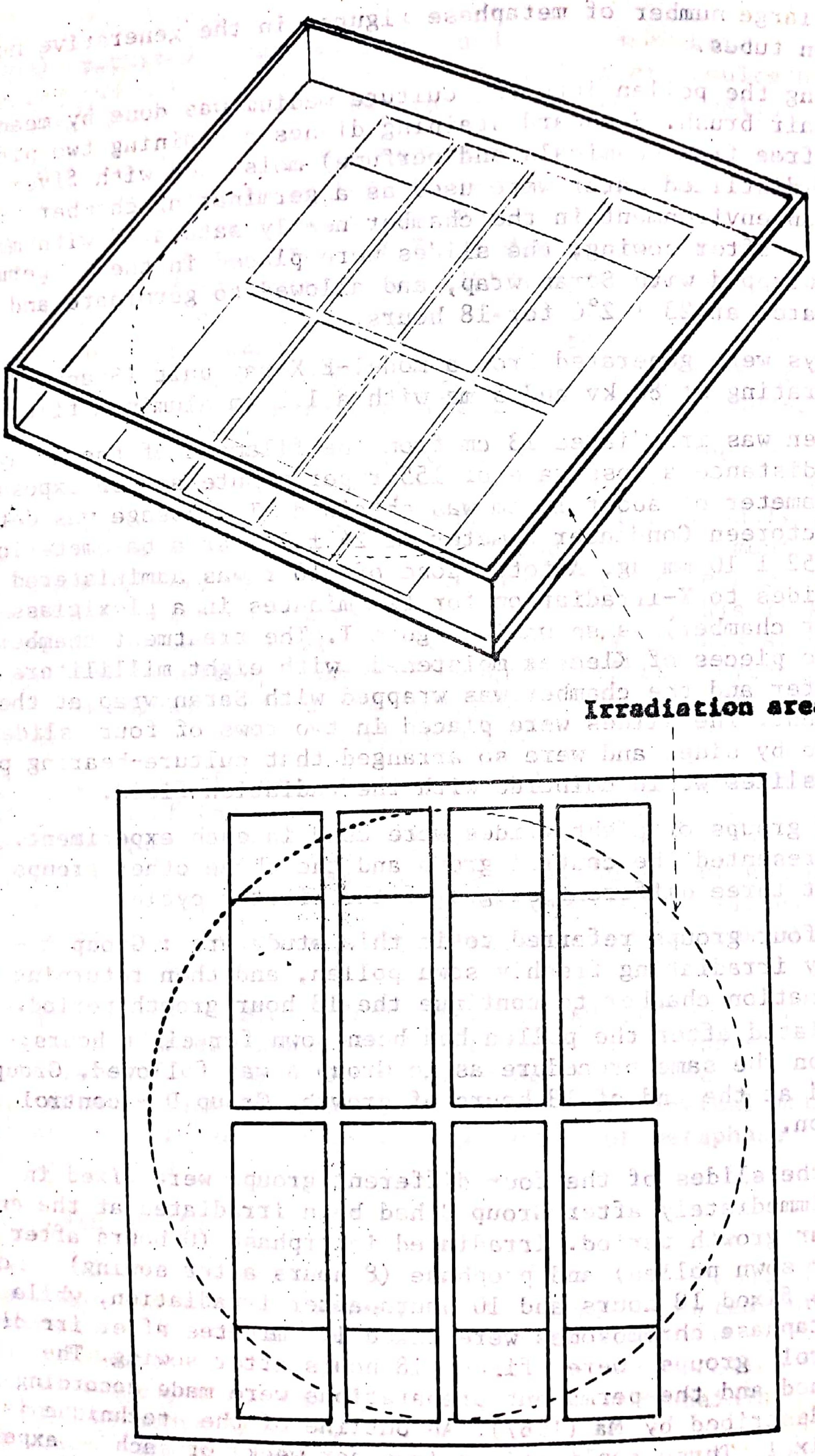

Figure I. Diagram showing the arrangement of the slides in plexiglass chamber during irradiation.

Chromatid aberrations were acored from 50 metaphase figures selected at random of each of the eight slides recorded in the group. The number and types of aberrations were examined and recorded in chromatid score sheets (Appendix II). The rate of aberration was calculated according to the accepted convention of taking chromatid and isochromatid breaks as one-hit events, and chromatid exchanges as two-hit events, and the data were expressed as breaks per 100 metaphase figures or cells.

III. OBSERVATIONS AND RESULTS

The mature pollen grains of *Tradescantia paludosa* were shed as binucleate cells; each had a generative nucleus and a vegetative or "pollen tube" nucleus. Their chromosomes were effectively doubled within two days (Newcombe, 1942) or three days (Bishop, 1950) prior to anthesis, as far as their vulnerability to X-radiation is concerned. At this stage the generative nucleus was considered to be in the late interphase or very early prophase (Brewbaker and Emery, 1962), prophase (Iwanami and Matsumura, 1963), G_2 (Savage, Preston and Neary, 1968) or early G_2 (Ma, Snope and Chang, 1971) as designated by the former investigators.

During germination or pollen tube development the vegetative nucleus became diffuse and degenerate, while the generative nucleus underwent the second division of pollen mitosis. The average time required for the generative nucleus to reach metaphase was 16 hours (Ma, 1967). Cultures of this study did not proceed beyond normal metaphase stage at the end of 18 hours of growth as indicated by the absence of ski-shaped, late c-metaphase chromosomes.

Since the chromosomes were effectively doubled at the time of treatment in all pollen for which data were obtained, chromosomal aberrations were rarely observed in metaphase figures.

Three types of chromatid aberrations were observed during the mitotic division in the pollen tube : chromatid breaks, isochromatid breaks and chromatid exchanges. The rare occurrence of centromere breaks, which were considered to be produced by single hits, were scored as chromatid breaks. Included as isochromatid breaks are : SU (sister chromatid unions), NUp (non-union in proximal portions), NUd (non-union in distal portions) and NUpd (non-union in proximal and distal portions). Two types of chromatid exchanges were observed : the symmetrical and asymmetrical interchanges. Most of these exchanges were symmetrical, and both symmetrical and asymmetrical interchanges were scored as chromatid exchanges. The very rare occurrence of chromatid rings was considered to be two-hit aberrations.

The number and frequency of aberrations in the three experiments are presented in Table I. Chromatid and isochromatid aberrations were calculated as one-break events, while exhanges were calculated as two-break events.

The Students' t-test was used to prove that the three experimental results were consistent. Interphase chromosome breakage events had probability values in the range of 0.30 to 0.70, prophase breakage events in the range of 0.30 to 0.70, and metaphase breakage events in the range of 0.30 to 0.90. The analyzed data are presented in Table II.

A chi-square test was used for the analysis of variance. The results are presented in Table III. It was found that there is a significant difference at the 0.05 probability level between treatments. By using the Duncan's multiple range test, it was found that there is no significant difference between the control and metaphase treatment at 0.05 probability level (degrees of freedom = 6). The results are presented in Table IV. From these data it was shown that there are significant differences in the other treatments (metaphase, prophase, interphase).

Table I.

Chromatid Aberrations in Pollen Tubes of *Tradescantia* Induced by X-rays (310r) at Interphase, Prophase and Metaphase Stages

Expt. ser- ies	Stages treated	No. & types of abberrations Cd*	Iso*	Exch*	No. of cells scored	Aber.freq. aber./100 cells	Net freq. aber./100 cells	Recombi- nation index
I	Interphase	92	385	58	400	148.3	145.8	0.122
	Prophase**	10	62	10	214	43.0	40.5	0.139
	Metaphase	44	0	0	400	11.0	8.5	0
	Control	3	7	0	400	2.5	-	-
II	Interphase	75	352	39	400	126.3	125.0	0.091
	Prophase**	16	78	2	320	30,6	29.3	0.021
	Metaphase	30	2	0	400	8.0	6.7	0
	Control	0	5	0	400	1.3	-	-
III	Interphase	61	478	45	400	157,3	148.5	0.083
	Prophase**	13	114	0	324	39.2	30.4	0
	Metaphase	67	13	0	400	20.0	11.2	0
	Control	4	31	0	400	8.8	-	-
Mean of experiments I, II, and III :								
	Interphase					143.9	139.7	0.099
	Prophase					37.6	33.4	0.053
	Metaphase					13.0	8.8	0
	Control					4.2	-	-

* Cd, Iso, Exch., represent chromatid break, isochromatid break and exchange, respectively.

** Presence of chromosome stickiness.

Table II.

Values of Probability for the Three Individual Treatments in Experiments I, II and III (after subtracting the control) as Analyzed with the t-test, at 0.05 Level of Probability

Treatment	Experiment I	Experiment II	Experiment III
Interphase	0.50 - 0.70	0.30	0.50
Prophase	0.30 - 0.50	0.50 - 0.70	0.50 - 0.70
Metaphase	0.90	0.30 - 0.50	0.30 - 0.50

Table III

The Analysis of Variance of the Four Treatments Within the Three Experiments

Treatment	Experiment I	Experiment II	Experiment III	Treatment Total	Treatment Mean
Control	2.5	1.3	8.8	12.6	4.2
Interphase	148.3	126.3	157.3	431.9	143.9
Prophase	43.0	30.6	39.2	112.8	37.6
Metaphase	11.0	8.0	20.0	39.0	13.0
Expt.total	204.8	166.2	255.3	596.3	49.7

Source of variance	Degrees of Freedom d.f.	Sum of Square SS	Mean Square MS	Observ. F	Required F 0.05	Required F 0.01
Total	11	38049.11				
Experiment	2	450.25	225.12			
Treatment	3	37349.26	12449.75	299.7*	4.76	9.78
Error	6	249.60	41.60			

* Significant at 0.05 level.

Tabel IV

Duncan's Multiple Range Test at 0.05 Level
(degrees of freedom = 6)

Treatment Means Compared	Relative Position of Mean in the Array.	Shortest Significant Range	Treatment Mean Difference	Conclusion (0.05 Level of Significance)
I - C*	4	13.5	139.7	I larger than C
I - M	3	13.3	130.9	I " " M
I - P	2	12.9	106.3	I " " P
P - C	3	13.3	33.4	P " " C
P - M	2	12.9	24.6	P " " M
M - C	2	12.9	8.8**	Insignificant

* I = interphase; P = prophase; M = metaphase; C = Control.
** Insignificant difference.

Treatment	Control	Metaphase	Prophase	Interphase
Mean	4.2	13.0	37.6	143.9

Chromosomal lesion or gaps were not recorded as induced aberrations or breaks, although they did occur. It was not always easy to distinguish between some of the chromatid deletions and lesions, especially on cells from irradiated prophase. If there were any connections or continuity along the axis of the damaged chromatid or centromere (long, thread-like connections between the "separated" chromatids, for example) the aberration was classified as a gap and was not included in the score for chromatid aberrations or breaks. Such gaps have been found in considerable numbers.

On rare occasions, some cells with ragged or shattered chromosomes have been observed in cells irradicated at prophase and interphase. The unusual damage shown in these cells makes the damage impossible to score in terms of the usual chromatid aberrations. These abnormal cells have been studied by Neary, Savage and Evans (1964) in *Tradescantia* pollen tubes. Such conditions were not found in irradiated metaphase.

Pycnosis, or stickiness of chromosomes, was observed in considerable numbers of cells irradiated at prophase. Pycnotic metaphase figures of this group were not included as aberrations in the data. Aberrations might have occurred in these pycnotic metaphase figures but they were not observable. Consequently the aberration rates of this group *indicated* in the data, were considerably reduced. This phenomenon had been observed earlier by a number of investigators. No pycnosis was observed in cells treated at interphase and metaphase stages.

Some observed types of chromatid aberrations and chromosome stickiness are presented in Figure II.

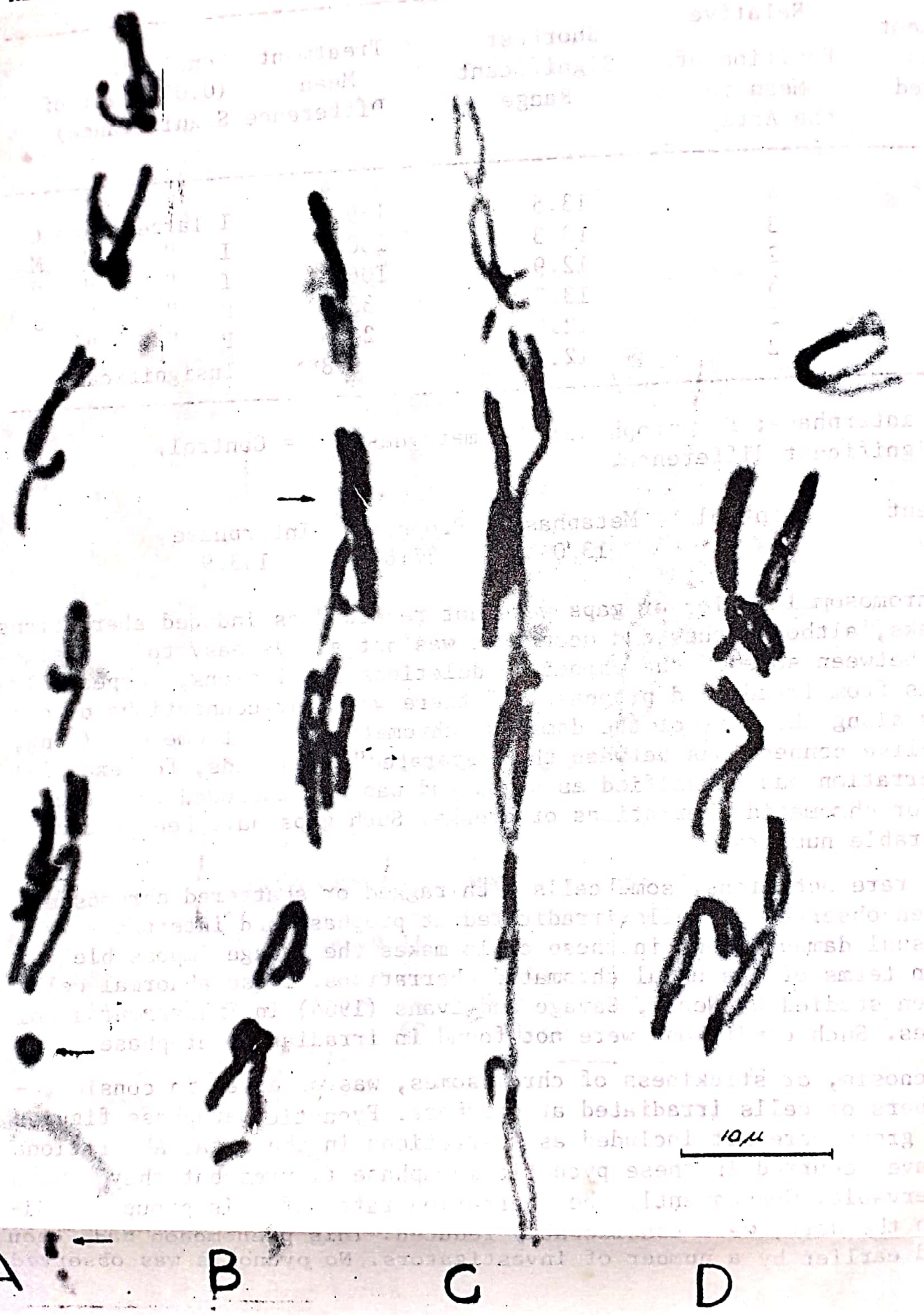

Figure II: X-ray induced chromatid aberrations and pycnosis. A. Ring (upper arrow) and chromatid break (lower arrow). B. Symmetrical interchange (arrow). C. Chromosome pycnosis (treated at prophase). D. Normal chromosomes.

IV. DISCUSSION

Pollen tubes cultured in an artificial medium offer an excellent system for the study of the cytological effects of radiation. The monolayered distribution of the pollen tubes on the lactose agar medium provide especially suitable material for the study of ultraviolet and other forms of radiation having low penetration; such forms of radiation cannot be used on multilayered tissues satisfactorily. The possession of only six large chromosomes, a low percentage of spontaneous aberration in Sax's clone-3 and a fairly synchronized population with regard to mitotic stage, are advantages for the quantitative and qualitative analysis of chromatid aberrations.

The types of chromatid aberrations induced in pollen or pollen tubes by X-rays are qualitatively indistinguishable from those induces by other ionizing or ultraviolet irradiation. Kirby-Smith, Sheppard, and Craig (1954) using fast neutrons, X-rays and 60 Co gamma rays, and Bailey (1963) using X-rays and ultraviolet rays for the irradiation of *Tradescantia paludosa* pollen found chromatid and isochromatid aberrations and chromatid exchanges. Evans, Neary, and Preston (1968) from their studies with *T. bracteata* Small. pollen found chromoseme rings besides those three types of chromatid aberrations, whereas Swanson (1940) reported only chromatid and isochromatid breaks from his X- and ultraviolet radiation studies on pollen and pollen tubes of *Tradescantia paludosa.*

My observations demonstrate that cells irradiated at interphase-G_2 stage resulted in three types of chromatid aberaations and chromatid rings, which are similar to those of the provious studies. Swanson (1940) reported some chromosome aberrations form his pollen studies, indicating that some of the chromosomes were single-stranded at a relatively earlier stage. This type of aberration could not be found in my studies, indicating that after desiccation all of the chromosomes of the mature pollen grains were affectively doubled at the time of irradiation. The presence of chromatid exchanges on irradiated prophase chromosomes in pollen tubes had also been reported by Neary, Savage, and Evans (1964) and work of previous investigators reviewed by Brewbaker and Emery (1962). Agreater number of chromatid exchanges had been found in irradiated interphase than prophase. This could be interpteted as meaning that in interphase the non-homologous chromatids are closer to each other in comparison with those in prophase. This condition resulted in a higher yield of chromatid exchanges in interphase in comparison with those found in prophase. In methaphase, however, no chromatid exchanges could be observed from my studies. The repelling forces between the chromatid strands at this stage might have prevented intra-arm exchanges, and the separation of the individual chromosomes as the nucleus passes down the pollen tube in a c-metaphase reduced the probability for recombination between the non-homologous chromatids. Thus, chromatid aberrations could be found only in irradiated metaphase. The presence of isochromatid aberrations in irradiated metaphase might be the result of spontanenous aberrations occurring earlier, since most aberrations found in the baseline control were of this type. This interpretation was based on the assumption that radiation could only break one metaphase chromatid at a time. The rare occurrence of chromatid rings, as shown in Figure II, would result from two independent hits in the same chromosome arm of the doublestranded interphase chromosomes.

As indicated in Table I, the frequency of aberrations produced by a given dose of X-rays depends upon the stage of the cell cycle irradiated. Based on the total number of breaks (aberration frequency) within the individual treatment (interphase, prophase, or metaphase), it was found from the means of the three experiments that interphase has the greatest aberration frequency, 139.7 breaks per 100 cells, followed by prophase with 33.4 breaks per 100 cells, and metaphase with 8.8 breaks per 100 cells. The mean of the baseline control yielded 4.2 breaks per 100 cells. A relatively high degree of breakage was found in the control of experiment III (8.8 breaks per 100 cells). This high degree of breakage in the control might be caused by some environmental factors, possibly by the presence of pollutants in the laboratory during the course of experimentation, or some other environmental factors. A similar discrepancy has also been reported by Ma, Snope, and Chang (1971) using the same experimental material.

From the t-test analysis it could be demonstrated that the three experiments were fairly consistant, since individual treatments showed normal deviations from the mean in these three experiments. The interphase breakage events had probability values in the range of 0.30 to 0.70, prophase in the range of 0.30 to 0.70 and metaphase breakage events in the range of 0.30 to 0.90.

Based on the aberrations frequency, it was found that metaphase chromosomes showed the least radiosensitivity. Similar results following X-radiation had also been reported by Sax and Swanson (1941) and Darlington and La Cour (1945) based upon experiments using *Tradescantia* microspores. Sax and Swanson (1941) suggested that the high concentration of nucleic acid at metaphase has a protective effect in preventing breakage of metaphase chromosomes, whereas Darlington and La Cour (1945) concluded that chromosomes in the condensed state are "either unbreakable by X-rays, or, if broken always undergo prompt restitution". In this connection it could be assumed that nucleoproteins would protect the DNA against radiation. Based on the studies of Mirsky and Ris (1947; 1951), the elementary chromosome fibril is composed of DNA-histone molecules linked together by nonhistone (residual) proteins, and both DNA and proteins would be essential for the structural integrity of the chromosomes. Bonner (1965) suggested also the possible fungtions of the various histones in stabilizing the DNA double helix. This various kinds of histones and residual proteins would form the protective proteinaceous sheath which enclosed the DNA double helix. By assuming DNA as the radiation target, as proposed by Bauer, Loring, and Kurnick (1965) and Dean, et al. (1969), this proteinaceous sheath would protect the DNA against the attack of the various free radicals formed by the ionizing radiations. Emmerson, et al. (1962) investigated in detail the chemical effects of free radical attacks on DNA, in particular base destruction and breakage of the phosphodiester backbone. From their studies using soluble nucleoprotein prepared from calf thymus they found that the presence of protein in DNA-portein complexes reduced the radiation damage by as much as 90 per cent as compared with pure DNA. In this relation, chromosome breakage as the end reaction or end product of DNA damage caused by ionizing radiation would be prevented or lessened by the presence of the proteinaceous sheath. The DNA damage due to the breakage of the phosphodiester backbone was also found by Dean, et al. (1969). The low number of breakage thus found in the relatively resistant metaphase chromosomes could be related to greater protection afforded the DNA against radiation, since at this stage the chromosomes are most compact and contain more protein (residual proteins) in comparison with the other stages. At interphase G_2, the chromosomes attained their minor coils, whereas in metaphase the chromosomes attained their minor and major coils and compacted together by the increase of proteins. In this relation, Swanson, Mertz, and Young (1967) stated that the residual protein content of metaphase chromosomes is about eight times that of their interphase counterparts. This condition was reflected in more resistant metaphase chromosomes, as compared to earlier stages.

Interphase with its extended chromosomes and less residual proteins would give more breaks, since at this stage the DNA is "loosely" protected. The more contracted prophase which would caontain more protein would therefore obtain more protection against radiation, and would produce a lower a lower number of breaks. These various degrees of protection would therefore have resulted in various rates of aberration induced by X-irradiation.

Kihlman (1966) related the peak sensitively of interphase-G_2 to the physiological changes in this stage which result in chromosome spiralization and contraction and the onset of division.

My results agree with the studies of Wolff and Luippold (1964) and Ma and Wolff (1965), who found a peak of sensitivity in interphase-G_2 and a decreasing number of chromatid aberrations as metaphase was approached.

Sidorov and Sikolov (1966) with their experiments on *Crepis capillaris* found anaphase, metaphase, and late prophase to be the stages of high sensitivity to radiation, and decreasing through early prophase, G_2 and G_1. These contradictory results might be interpreted, in part, as the result of the different method and criteria used to judge the radiosensitivity. In their studies they used asymmetrical chromosome aberration frequency at metaphase of the second post-treatment mitosis of colchicine-induced tetraploid cells. My results are also in disagreement with the studies of Reynolds (1940) on fungus fly oocytes, Whiting (1945) on wasp oocytes, Conger (1947) using onion root-tip cells, Sparrow (1948; 1951) using *Trillium* pollen mother cells. Bishop (1950) working with *Tradescantia* microspores, Davidson (1958) using *Vicia faba* roots, and Marakami (1967; 1969) with his studies on silkworm eggs. These investigators who claimed metaphase to be a stage of high radiosensitivity, used the double scoring method for analyzing the radiation effects. The aberration frequency or other criteria for measuring the radiosensitivity were analyzed at metaphase of the second post-treatment mitosis or meiosis, or after the chromosomes had been passed through the intervening interphase. In this relation Sparrow (1951), Crouse (1954) Davidson (1958) and Grosh (1965) stated that no breaks were visible immediately after irradiation and the high potential breaks in metaphase chromosomes would be recovered during the next division.

In the present studies, however, this double scoring method could not be applied since the material used did not undergo further division.

Zermeno and Cole (1969) who assumed chromatin as the radiation target, suggested that the high radiosensitivity of metaphase cell lies in the peripheral distribustion of the radiosensitive material, while in radioresistant interphase the distribution of interphase radiosensitive material is diffuse. On the other hand, Dewey and Thompson (1967) using the same material (Chinese hamster cells) found a peripheral distribution of Radiosensitive chromatin during interphase.

Chromosome pycnosis or stickiness in the irradiated prophase has also been observed by various investigators. In *Tradescantia* studies, Sax and Swanson (1941) found stickiness between 4-12 hours after irradiation, Beatty and Beatty (1954) after 4 hours, Ma and Wolff (1965) found stickiness before 10 hours. Generalova (1969) using roots of *Crepis capillaris* found maximum stickiness 2 hours after irradiation, and no stickiness could be observed after 4 hours. Casarett (1968) claimed that this phenomenon, in general, is a result of partial dissociation of the nucleoproteins and an alteration in their pattern of organization. This stickiness is generally assumed to be a recoverable or reversible process (Beatty and Beatty, 1955;Casarett. 1958; Wolff, 1968). In the present study, the absence os stickiness in the irradiated metaphase chromosomes was an exceptional case, since most of the former investigators found chromosome stickiness in *Tradescantia* microspores and *Vicia* roots if the irradiated materials were observed within 10 hours period after irradiation.

V. CONCLUSIONS

From the above results it can be concluded that the generative nucleus in mature pollen of *Tradescantia paludosa* was in interphase-G_2. Based on the aberration frequency, it was found that metaphase chromosomes have the lowest radiosensitivity (8.8 breaks per 100 cells) when compared to prophase (33.4 kreaks per 100 cells) and interphase (139.7 breaks per 100 cells) chromosomes. The change in radiosensitivity was accompanied by changes in the number of chromatid exchanges induced. The absence of exchange in metaphase chromosomes is presumably caused by changes in the spatial relationship among the broken ends of non-homologous chromatids and inability of these chromatids to form recombinants. Pycnosis was observed in irradiated prophase, but not in irradiated interphase or metaphase chromosomes in the pollen tubes.

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APPENDIX I

OUTLINE OF SLIDE PREPARATION FOR POLLEN TUBE CHROMOSOMES

1. Collect pollen at 11 : 00 a.m. (summer)
2. Desiccate 11 : 00 - 15 : 00
3. Sow pollen onlactose-agar medium and colchicine (± 39°C) lactose - 3 g, agar - 0.375 g, water 17 ml, colchicine 5 ml (0.2% solution).
4. Treatment.
5. Place growth chamber in incubator at 23 ± 2°C, for 18 hours.
6. Fix 9:00 - 10:00 a.m. (next day) in :
   chromic acid 0.7 g, glacial acetic acid 0.5 ml, water 100 ml :
   60 minutes.
7. Wash in cold 1 N HCL.
8. Hydrolyze in hot 1 N HCL 60°C - 6 minutes. (heat HCL to 68°C in order to obtain 60°C after immersion of the slides).
9. Hot water treatment 65°C - 1 minute. (heat water to 80°C in order to obtain 65°C).
10. Cold water wash - 1 minute.
11. Flush off medium (under running water).
12. Apply cover glass.
13. Press on slide dryer (about 80°C).
14. Dry ice treatment (15 minutes or more).
15. Remove cover glasses with razor blade.
16. Wash in tap water 5 minutes, followed by distilled water 5 minutes.
17. Stain in Feulgen (20°C - 6 minutes).
18. Wash in running tap water (12 minutes).
19. Differentiate in 45% acetic acid (10 minutes).
20. Dehydrate in 70% alcohol (5 minutes).

21. Dehydrate in 95% alcohol (15 minutes or more).
22. Mount in Euparal.
23. Press under lead block for one day.
24. Dry for 4 days.

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