THE EFFECTS OF ULTRAVIOLET AND X-RAY TREATMENT ON THE POLLEN TUBE CHROMOSOMES OF TRADESCANTIA

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THE cytogenetic interest in ultraviolet radiation resides in the hope that the qualitatively discriminating nature of this type of radiant energy will yield still further evidence as to the structure and behavior of the hereditary mechanism. It is now known that ultraviolet, like X-rays, can produce gene mutations and chromosomal changes; it is likewise known that, unlike X-rays, ultraviolet is absorbed in a definite and specific manner by the proteins and nucleic acids which constitute, as far as we are aware, the physical basis of the gene. The integration of these two kinds of investigation, the one biological and the other physico-chemical, should, in time, yield a more informative mass of data concerning the relationship between absorption on the one hand and the genic or chromosomal change on the other than the less discriminating ionizing types of radiation.

The difficulties encountered in securing and measuring the effective penetration of ultraviolet make it necessary to use a variety of organisms for genetical and cytological analyses. The studies of STADLER and SPRAGUE (1936), STADLER (1939), and MULLER and MACKENSIE (1939) have been on effects visible only after the course of many cell generations, and on those which in diploid cells are genetically viable and hence transmissible. It has been shown that such studies can be confirmed and furthered by an analysis of the more immediate effects of ultraviolet through use of the pollen tube technique (SWANSON 1940a, b), which permits an analysis of both viable and non-viable chromosomal changes before they can be eliminated through the processes of mitotic division.

MATERIAL AND METHODS

Plants are more adaptable to ultraviolet investigation than are animals because of the comparative ease of reaching the germinal material with the genetically effective wave lengths. In this study a diploid clone of *Tradescantia paludosa* Anderson & Woodson was used exclusively, the chromosomes of the generative nucleus, dividing in pollen tubes grown on an artificial sugar-agar-gelatine medium, furnishing a convenient source of material (Plate 1, A). The technique has been previously described in detail (SWANSON 1940b). In this study it was modified to the extent of re-

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placing the acenaphthene with a 0.001 percent aqueous solution of colchicine, this solution being incorporated in the medium.

The generative nuclei were irradiated two hours after the pollen grains had been dusted onto the agar medium. This, it was found, was a rather necessary procedure. Tradescantia pollen, unlike that of other plants such as maize, has a tendency to clump at the time of shedding and hence cannot be spread evenly in a monolayer. To avoid the screening effect brought about by clumping, it was necessary to delay radiation until the pollen tubes has grown out onto the surface of the medium and the generative nucleus had passed from the grain into the tube. Germination took place in fifteen minutes, and by two hours' time the majority of the tubes were in the correct stage for exposure. This treating schedule was adhered to except where noted.

Three ultraviolet sources were utilized in this study: (1) A high voltage discharge tube (Hanovia Sc-2537) operating at 5000 volts. Eighty-five percent its of ratiation is of wave length 2537. A quartz water cell was used in conjunction with this light to filter out wave length 1849 which, although present in only slight amounts, was found to be particularly injurious to the growth of pollen tubes. At 20 cm from the tube, this light gave off approximately 1000 ergs/mm^2/minute. Treating distances of 10 and 20 cm were used, but the data were lumped (table 1) since the intensity factor

TABLE 1

Chromatid deletions induced by ultraviolet. High voltage discharge tube (83 percent 2536 Å).

EXPOSURE TIME	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS
15 sec.	1124	8	.65 ± .24
30 sec.	2904	34	1.17 ± .19
1 min.	6426	174	2.71 ± .20
2 min.	4314	238	5.52 ± .34
4 min.	936	95	10.15 ± .98

was found to be non-operative. (2) A monochromator designed for the treatment of maize pollen (UBER and JACOBSOHN 1938, UBER 1940). Only wave length 2537 was used, and doses up to 3150 ergs/mm^2 were given. Higher doses yielded too few analyzable figures per slide. (3) A mercury arc, fitted with 1/80 M diphenyl filters so as to permit only the passage of wave lengths 2967 and 3022, and longer. At 12.5 cm from the arc, the treating distance, the energy values were 7000 ergs/mm^2/minute for the shorter wave length and 21,000 ergs/mm^2/minute for the longer one. These latter figures represent approximations only.

ULTRAVIOLET INDUCED ABERRATIONS

A previous study of about fifty aberrations had indicated that only single chromatid deletions resulted from the direct treatment with ultraviolet of the prophase chromosomes in the pollen tubes of Tradescantia (SWANSON 1940a). These deletions, so far as could be determined cytologically, were strictly terminal in nature. This conclusion has been made more certain by a study of more than 700 additional deletions (Plate 1, fig. B, C, D, E), all of which appear to be truly terminal. These data are in agreement with those obtained in maize where STADLER and his coworkers (STADLER 1939, SINGLETON 1939, SINGLETON and CLARK 1940) have similarly found from pachytene studies that insofar as they could determine, the deleted portion was invariably terminal. In the pollen tubes a few instances were found of sister chromatids being broken at sensibly identical loci, but in view of their scarcity, and since such sister breaks have been found as frequently in control material, it seems most probable that they are of spontaneous origin. A number of chromatid dicentrics and exchanges were also found in irradiated cells, but these, again, were no more frequent than in the controls and revealed no relation to dosage, so they must likewise be considered of a spontaneous nature.

The induced terminal deletions showed all gradations from free fragments to achromatic lesions. Most X-ray studies have disregarded aberrations of this sort because of the difficulties involved in accurate scoring, but since with ultraviolet treatment only single chromatid deletions are produced, they must be considered if any breakage data are to be obtained. Since the pollen tube technique does not permit analysis beyond the immediate cell division irradiated, it is uncertain as to whether achromatic lesions constitute true deletions. In order, therefore, to be as objective as possible in scoring, only those aberrations revealing a distinct separation between the two broken ends were considered as true deletions.

Chromatid deletions in Tradescantia pollen tubes, whether induced by X-rays or ultraviolet or arising spontaneously, are rarely dislocated from their original position. As a rule, they remain fixed in place (Plate 1, fig. C, E), held, probably, by a matrix which, as will be shown later, becomes abnormally swollen and prominent under the action of the shorter ultraviolet wave lengths. Only infrequently is the deletion moved out of place (Plate 1, fig. B, D).

Do these data agree with previous findings in other organisms? The most extensive ultraviolet investigations have been carried out by STADLER and SPRAGUE (1936) and STADLER (1939) on endosperm deficiencies in maize. These deficiencies, since they are largely of the fractional variety,

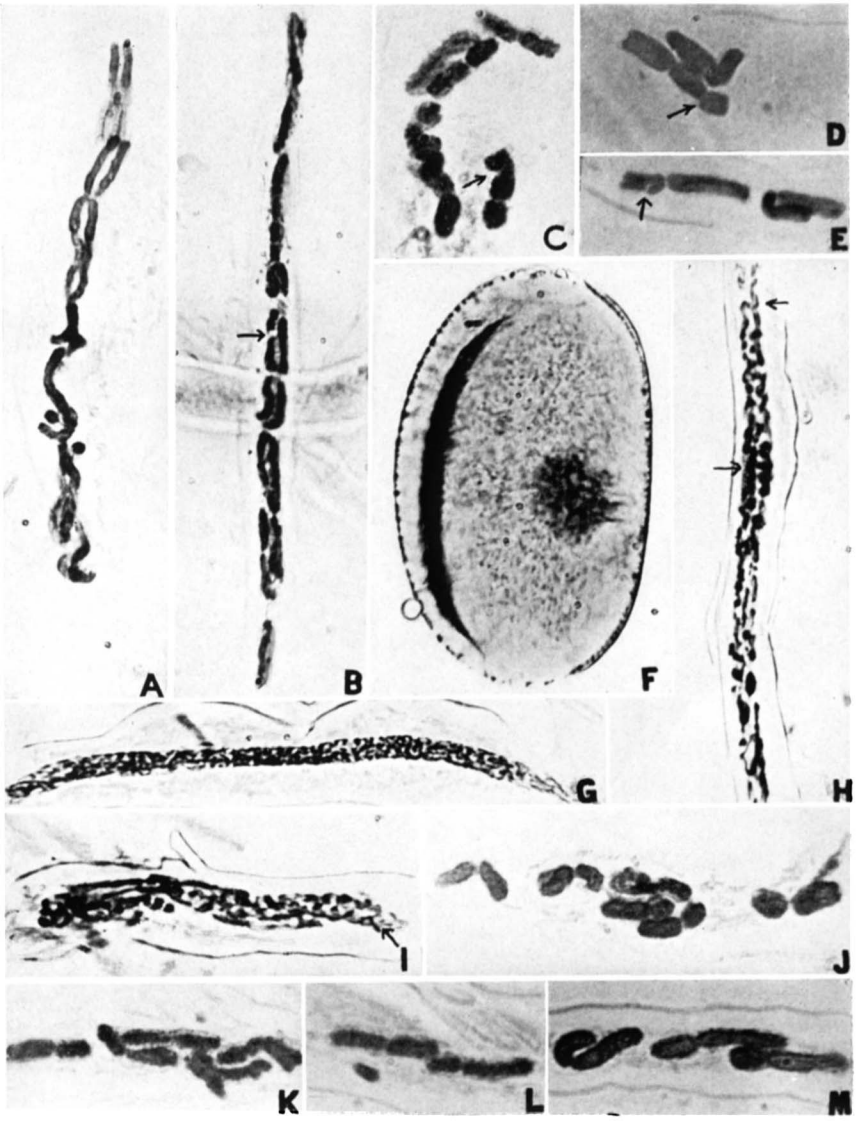
may be best explained by assuming that they represent chromatid losses comparable to those produced in Tradescantia pollen tubes. The pollen tube technique does not permit one to demonstrate that the chromatid deletions will result in segmental deficiencies of the kind shown by SINGLETON (1939) and SINGLETON and CLARK (1940), but it seems reasonable to assume that they will. STRAUB (1941) has also reported deletions (Stuckverlüste) in *Gasteria trigonia*, following treatment of mature pollen grains. These apparently terminal deletions, which STRAUB found to be not infrequent, were losses in somatic tissue, a condition which STADLER (1939) finds to be of rare occurrence in maize. An explanation for this difference in somatic losses might logically be sought in the differential capacity of the plants to survive chromatin losses, but STADLER has made it quite clear that some other explanation must be sought to clarify the situation in maize.

In addition, both STADLER and STRAUB have reported the occurrence of translocations following ultraviolet treatment. STRAUB'S data seem to indicate that the translocations (five in number) were of the reciprocal type, while the three reported by STADLER were all deficiency-translocations. That the absence of translocations in the dividing generative nucleus cannot be ascribed to an insufficiency of breaks available for illegitimate reunion is evident from the data in tables 1 and 5, where X-ray and ultraviolet deletions may be compared. However, the immovability of the ultraviolet induced deletions, due possibly to the inability of ultraviolet to break through the matrix (MULLER 1940) and to the stability of such broken ends (McCLINTOCK 1941), presages an absence of translocations at least until the release of the deletion, which in the case of the sperm nucleus would occur at the time of fertilization. This delayed formation of illegitimate reunions, following ultraviolet treatment, would parallel the delayed production of gene rearrangements in Drosophila following X-ray treatment (MULLER 1940, KAUFMANN 1941). It is, of course, possible that the occasional translocation found in the pollen tubes following irradiation is of an induced nature, but since the frequency is not above the spontaneous rate, nothing definite can be said regarding their rate of occurrence.

In Drosophila, MULLER and MACKENSIE (1939) found no chromosomal changes, either losses or rearrangements. This is perhaps due more to the lethality of even minute losses than to total absence of such changes.

RELATIONSHIP BETWEEN DOSAGE AND THE FREQUENCY OF BREAKS

STADLER and UBER (STADLER 1939) have shown that for specific endosperm deficiencies, where the induced changes are most likely the result of chromosome or chromatid deletions, the dosage curve is essentially a linear one with a leveling-off at the higher doses due to saturation effects. This



EXPLANATION OF PLATE 1

Photographs of the generative nucleus of Tradescantia.

A.—Normal metaphase.

B–E.—Ultraviolet induced deletions.

F.—Mature pollen grain showing crescent shaped generative nucleus and the diffuse tube nucleus.

G.—Generative nucleus in pollen tube two hours after germination.

H.—Generative nucleus four hours after germination. Lower arrow points to incipient somatic coils; upper arrow points to region where chromosome is split.

I.—Generative nucleus four hours after germination. Arrow indicates split chromosome.

J.—Greatly contracted chromosomes following treatment with wave length 2536; matrix not visible.

K–L.—Show development of matrices following treatment with wave-length 2536.

M.—Late prophase; matrix just beginning to show.

implies the effectiveness of single quantum absorptions. To test this further, a series of experiments were carried out to determine the dosage curve for directly induced deletions. A leveling-off should not be expected, since all deletions would be readily detected.

Table 1 and figure 1 summarize the data derived from a series of dosage

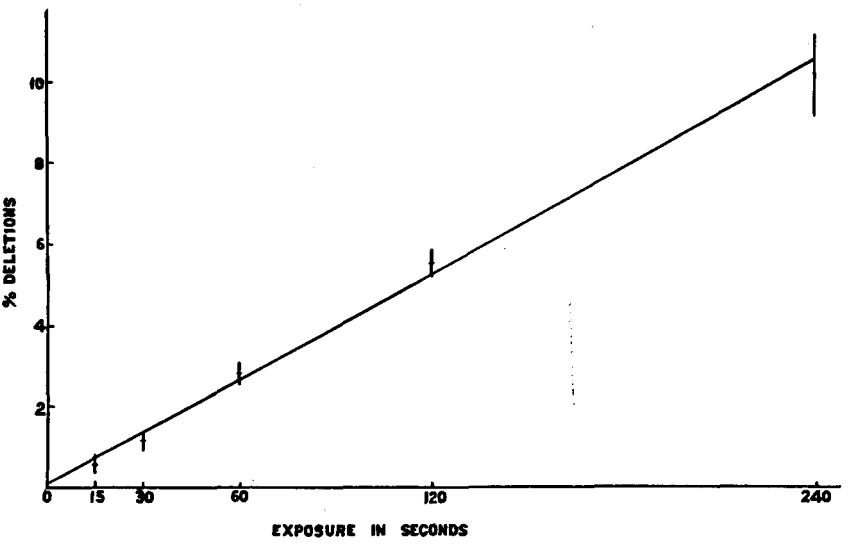


FIGURE 1.—The dosage-break relationship for simple chromatid deletions in the pollen tube chromosomes of Tradescantia at wave length 2537.

experiments employing the high voltage discharge tube. There can be little doubt of the linearity of relationship. This conclusion is further substantiated by data derived from a series of treatments using monochromatic light (wave length 2537). These are given in table 2. The errors are large,

TABLE 2

Chromatid deletions induced by ultraviolet. Monochromator. Wave length 2536.

ERGS/MM^2	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS
485	2472	19	0.77± .17
525	1008	10	0.99± .31
825	1620	7	1.66± .31
1012	282	6	2.16± .86
1050	1416	21	1.48± .32
1650	462	12	2.60± .74
2024	384	8	2.08± .73
3000	594	27	4.54± .85
3036	312	20	6.41±1.38
3150	1020	52	5.10± .69

due to the small number of observations, but the relationship is approximately linear. Can one assume, however, that the same relationship holds throughout the genetically effective spectral range, particularly in the region of the genetically weaker wave lengths? This was tentatively tested by treatments with light composed of the last two effective wave lengths (a mixture of wave lengths 2967 and 3022). The data (table 3) are meager

TABLE 3

Chromatid deletions induced by ultraviolet. Mercury arc. Mixture of wave length 2967–3022.

EXPOSURE TIME	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS
15 sec.	1410	14	.99± .26
30 sec.	1182	15	1.27± .32
1 min.	1122	13	1.16± .32
2 min.	1200	31	2.58± .45
4 min.	462	16	3.46± .85
5 min.	216	11	5.09±1.49

and not entirely conclusive, but within the dosage used, the linearity of effect holds. The relative ineffectiveness of the longer wave lengths is indicated by a comparison with monochromatic 2537. A dose of 3000 ergs/mm^2 at wave length 2537 yields approximately 5 percent deletions. To produce the same number of deletions with a mixture of wave lengths 2967 and 3022 requires a dose of approximately 35,000 ergs/mm^2 of the former plus 100,000 of the latter. It should be emphasized, however, that these energy data are only approximate.

A comparison of the above data with those of STADLER (1939) shows that the endosperm deficiencies per unit dose of wave length 2537 are much greater in number than the terminal deletions in the generative nucleus. However, the differences in nuclei, stage of mitotic development, and methods of detection make it difficult to carry the comparison further.

THE LOCALIZATION OF CHROMATID DELETIONS

X-ray analyses of induced aberrations have revealed that the breaks are not randomly distributed along the chromosome arms. Some regions appear to be more susceptible to breakage than others. In Tradescantia microspores both X-ray and spontaneous breaks tend to have a greater proximal frequency (SAX and MATHER 1939, GILES 1940), due, according to these investigators, to the presence of a greater torsional strain imposed upon this region by the coiling mechanism. STRAUB (1941) has reported a similar distribution of breaks following both X-ray and ultraviolet treatment. X-radiation of the X chromosome of Drosophila revealed a more random break distribution, but still with a significantly higher increase

over expectancy in the heterochromatic proximal portion (BAUER, DEMEREC, and KAUFMANN 1938). KAUFMANN (1939, 1941), in a more intensive study, found that a differential break frequency obtains when particular regions are taken into consideration and concludes that this is because of the localized presence of heterochromatin. In Drosophila and Tradescantia, secondary factors, such as propinquity, movement, etc., probably play effective roles in obscuring the original frequency and distribution since the aberrations scored depend upon the existence of two breaks followed by illegitimate reunion, whether sister or non-sister. The relative absence of translocations following ultraviolet treatment indicates that illegitimate reunions are at a minimum or occur not at all in the pollen tubes. The observed break frequency should therefore more nearly reflect the original frequency, considering the stability of the broken ends so induced (MCCLINTOCK 1941).

Table 4 summarizes the data compiled on the localization of simple

TABLE 4

Localization of chromatid deletions.

REGION OF ARM*	1	2	3	4	5	TOTAL CHR.
UV (2536 Å)†	62	109	166	134	108	579
X-ray (dry pollen)	18	23	20	35	25	121
X-ray (germ. 1¾ hrs.)	20	26	45	49	42	182

* Proximal region of arm = 1; distal region = 5.

† These deletions from experiments using monochromator (2537 Å) and high voltage discharge tube (85% 2537 Å).

chromatid deletions both for ultraviolet (wave length 2537) and X-rays. The X-ray data are included for the sake of comparison and because such deletions do not depend upon subsequent reunion for their permanence and expression and should therefore reveal an original distribution. X-ray treatment of the generative nucleus in the pollen grain showed the breaks to be more or less at random, χ^2 revealing no significant deviation at the 5 percent level.

On the other hand, treatment of the generative nucleus in the pollen tube, whether with ultraviolet or X-rays, produced a greater frequency of deletions in the medial and distal regions of the chromosome arms. Calculations for χ^2 showed these departures from randomness to be statistically significant, P being less than .01. These data are directly opposite to those reported in Tradescantia microspores for more complex breaks (SAX and MATHER 1939, GILES 1940), and in Gasteria for deficiencies (STRAUB 1941). The failure of the dry pollen X-ray series to reveal other than a random distribution may be due in part to the fact that the sample

is too small to show a significant departure from randomness, since on a percentage basis the distribution is not too far different from that in the ultraviolet series, but it may also be due to the lack of secondary factors, such as torsional strains, in the passive generative nucleus prior to its passage into the pollen tube. It should be emphasized at this point that the above considerations are based on the assumption that the degree of contraction of the somatic chromosomes between prophase and metaphase is equal in all regions. However, it is recognized that a localization of breaks at metaphase can occur from a random break distribution at prophase if, during the coiling cycle, a particular region of the chromosome contracts to a greater extent than the remainder of the chromosome, a condition known to exist, for example, in the B-chromosome of maize. On the other hand, the fact that in a single genus, Tradescantia, dissimilar localizations of breaks have been found would seem to suggest that secondary factors of some sort or another are operative and that these factors may not be the same in different types of cells. As reported in a later section, the ultraviolet treatment causes a marked shortening of the chromosome, which fact might tend to obscure or accentuate a localization of breaks, but since the X-ray series (germ. $1\frac{3}{4}$ hours) follows a similar trend, it would appear that a differential contraction factor cannot be used to explain the observed distributions.

CHROMATID BREAKAGE IN RELATION TO PROPHASE DEVELOPMENT

Experimental results have consistently shown that dividing cells are more susceptible to X-rays than are those in a resting condition. Investigators, however, differ as to the most susceptible period in the cycle of cell division insofar as chromosomal breakage is concerned.

In the pollen tube it is possible to study rather accurately the effect of radiation on different stages of nuclear development by subjecting the generative nucleus to various forms of radiant energy at stated intervals after the dry grains have been dusted on the agar medium. The regularity of germination and of prophase development of the generative nucleus under artificial culture conditions permits and justifies such a procedure. In this study both X-ray and ultraviolet treatments were given.

In the X-ray series, treatments were given to the dry grains just before they were germinated and to the generative nuclei at the 2-hour and the 4-hour periods after germination. The chromosomes in the pollen grain, judging on the basis of chromatid breaks, are effectively split at least a day, and possibly two, prior to anthesis. X-ray-induced chromsome breaks had been previously reported as a result of raying mature pollen grains, and it had been concluded that all the chromosomes in the generative nucleus were not split at the time of anthesis (SWANSON 1940a, SAX and SWANSON

1941). More recent experiments show that this conclusion can no longer be considered valid, and any chromosome break showing itself in the pollen tube must be looked upon as of spontaneous origin.

The data summarized in table 5 leave no doubt that the generative

TABLE 5

*Chromosome aberrations induced by X-ray treatment. Dose 123.2 r.**

CONDITION AT TIME OF TREATMENT	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS	DICENTRICS	EXCH.	PERCENTAGE OF TOTAL BREAKS
Dry pollen	1950	46	2.36± .34	70	15	7.48± .59
Germ. 2 hrs.	1806	181	10.00± .70	49	40	21.70± .97
Germ. 4 hrs.	456	74	16.22±1.72	11	13	24.34±2.00

* Dose given in 1 minute.

nucleus, when in the pollen grain, is considerably more resistant to X-rays than is the same nucleus two or four hours later after it has entered into the pollen tube. This is in agreement with the previous data of SAX and SWANSON (1941). Susceptibility increases as prophase advances if one considers that the nucleus enters prophase as it passes down the tube, a likely condition since the threads become progressively more chromatic and distinct (Plate 1, compare F with G, H, and I). This increased susceptibility is due largely to a continued increase in the number of terminal chromatid deletions, for it can be seen that a fall in the percentage of chromatid dicentrics and exchanges is found at the 4-hour period following a rise at the 2-hour period. The ratio of dicentrics to exchanges also varies, going from a 4.5:1 to a 1:1 ratio as prophase advances.

When successive prophase stages were treated with ultraviolet, a somewhat different situation obtains. These data are given in table 6. The dos-

TABLE 6

Ultraviolet induced deletions at successive prophase stages.

HOURS AFTER SOWING	TOTAL CHR.	DELETIONS	PERCENTAGE DELETIONS
2	1728	92	5.32±.54
3	1404	57	4.06±.52
4	1020	26	2.55±.49
5	1134	13	1.14±.31
9	1236	7	.56±.21
11	576	2	.35±.24
13	1002	2	.20±.14
15	1470	3	.21±.12
Control	2772	8	.28±.10

age (½ minute exposure, 10 cm treating distance, using high voltage discharge tube) was one which yielded an appreciable percentage of deletions but which did not seriously retard the growth of the pollen tubes. Susceptibility to ultraviolet followed a steady and continuous decline until at eleven hours after germination there was no further production of detectable chromatid deletions due to irradiation (the spontaneous rate is around 0.2 percent). The greatest drop, however, was found in the early prophase stages.

The identical conditions of the two experiments make it possible to compare, in respect to prophase susceptibility to breakage, the effects of X-rays and ultraviolet on the pollen tube chromosomes during the 2- to 4-hour stage after germination. The increase in percentage of deletions following X-ray treatment (table 5) is in direct contrast to the decline following ultraviolet treatment (table 6), further emphasizing the genetically qualitative difference between these two types of radiation (STADLER 1939). Whether a continued increase in deletions following X-radiation would result in later prophase stages is as yet undetermined, although SAX'S (1941, table 1) data on Tradescantia microspores would indicate that this might be expected.

The work of SAX (1941), SAX and SWANSON (1941), KAUFMANN (1941), and others indicates that the sensitivity of cells and chromosomes to X-rays, based on breakage data, can best be accounted for on the basis of chromosome movements, propinquity of breaks, and rapidity of nuclear changes, but it is evident that these factors must play little part in determining the effect of ultraviolet treatment since a decline rather than a rise in breakage percentage must be explained. Although the data furnish no clue to the problem, it seems likely that internal physical changes in the chromosome, the nature of which are unknown but which would effect absorption, determine the reactivity of the chromosome.

THE EFFECT OF SHORT AND LONG WAVE LENGTH RADIATION ON THE MATRIX AND ON CHROMOSOME LENGTH

Apart from the production of chromatid deletions, short wave length treatment (2537 Å) has a very noticeable effect on the length of the chromosome. The greater the dose, the more contracted are the chromosomes. Under control conditions, Tradescantia pollen tube chromosomes generally possess about twenty somatic coils at metaphase, but at doses of 3000 ergs/mm^2, a nearly lethal dose at monochromatic 2537, the number of coils was reduced to seven to ten per chromosome. The width of the chromosome gyre was correspondingly increased. The shortness of the chromosomes was striking (Plate 1, C and J) compared to the considerable length

of the normal ones (Plate 1, A). Apparently, the effect of short wave length radiation is to increase despiralization of the coiled chromonema (SWANSON 1942), since the chromosomes at the time of radiation have already begun to coil.

Accompanying the shortening of the chromosome length, and possibly correlated with it, is the marked appearance of the matrix. This structure, rarely seen so clearly under normal conditions, takes the form of a transparent hyaline mass surrounding each chromosome (Plate 1, K and L). The matrix becomes visible for the first time at middle or late prophase (Plate 1, M) and always includes both chromatids, usually holding them so closely approximated as to simulate a single coiled structure. Frequently the chromosome matrices fuse to form a confluent mass surrounding several chromosomes, and under conditions of heavy short wave length radiation, appear to be unable to divide into chromatid matrices as would normally occur. A few of the nuclei, even after heavy doses, lack the visible appearance of the matrix, but these likewise have the shortened length and juxtaposed chromatids (Plate 1, J). This would appear to indicate that ultraviolet can cause a marked contraction of the chromosome without affecting the matrix, a condition which would mean that chromosome contraction is a phenomenon apart from, and not dependent upon the behavior of the matrix. A change in the internal condition of the chromosomes, brought about by the short wave length radiation, results in an accentuated shortening. On the other hand, it may be that the matrix is present but not visible.

Such a behavior does not take place when the longer wave lengths (a mixture of wave lengths 2967 and 3022) are used, even though the dosage in ergs may be many times as great. The chromosomes are normal in appearance. There is no marked shortening of the chromosomes, the matrix is not visibly affected, and separation of the two chromatids readily takes place. It is probable that the matrical substance absorbs highly in the short wave length region, this absorption setting up a chemical reaction or change which leads ultimately to an abnormal contraction of the chromosome and a marked appearance of the matrix.

It is too early as yet to say much concerning the nature of the absorbing components of the matrix. However, SCHULTZ (1941) has shown that the matrix of the salivary gland chromosomes of Drosophila is readily digested away by ribo-nuclease, indicating that this structure depends upon the ribo-nucleic acid linkages for its integrity. It is possible that the ribonucleic acids, absorbing highly in the short wave length regions, may be responsible for the striking effect of the short wave lengths on the chromosomes.

SUMMARY

In addition to extending information on the capacity of ultraviolet to produce simple breaks in pollen tube chromosomes, the present study has brought out the following new evidence:

The frequency of chromatid breaks induced by ultraviolet at 2537 Å is directly proportional to dosage. A similar situation appears to hold for the longer wave lengths (2967 and 3022), but the scantiness of the data permit of no definite conclusion.

A non-random distribution of chromatid breaks was found when nuclei in the pollen tubes were treated with ultraviolet and X-rays. Breaks were more frequent in the medial and distal regions, contrary to that previously reported in Tradescantia and Gasteria.

A delayed series of ultraviolet treatments show that the prophase chromosomes become progressively more resistant as judged from breakage data. In the 2- to 4-hour period after germination, the chromosome appears to become more susceptible to X-ray breakage, but with ultraviolet, the reverse was true.

Following heavy doses of short wave length ultraviolet treatment at prophase, the metaphase chromosomes showed a marked shortening accompanied by the appearance of a hyaline sheath surrounding them. Longer wave lengths produced no such changes.

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