X-RAY AND ULTRAVIOLET STUDIES ON POLLEN TUBE CHROMOSOMES. I. THE EFFECT OF ULTRAVIOLET (2537 Å) ON X-RAY-INDUCED CHROMOSOMAL ABERRATIONS[1]

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Received June 4, 1943

INTRODUCTION

THE direct approach to the problem of the mechanism of chromosome breakage and reattachment—namely, an analysis of the end results following radiation—has not as yet yielded a wholly satisfying answer, although our understanding of the limitations within which the mechanism operates has been considerably broadened, and its complexity has been recognized. An indirect approach has been made by determining the effect on breakage of varying the environmental conditions before, during, and after radiation. MICKEY (1938), SAX and ENZMANN (1939), and FABERGÉ (1940) have demonstrated that high temperatures reduce the total observed X-ray breakage, although MICKEY'S results seem doubtful in view of the more recent data of MULLER (1940). The plant data are explained by SAX and ENZMANN as resulting from an increased rate of restitution, thus leaving fewer broken ends available for reattachment. More recently SAX (1943) has shown that centrifuging during radiation increases the number of breaks; this, he feels, is due to the increased stresses placed on the chromosome during the period of breakage and reattachment.

With the recognition that a series of canalized events must bridge the gap between the initiating ionization, which is on an atomic level, and the realization of the complete break and its later reattachment (or restitution), which by comparison are on a macroscopic level (DELBRÜCK 1940), the indirect method of approach to this problem assumes considerable importance. By breaking down the series of events into separate units (at present only a theoretical possibility), a more thorough study could be made of the nature of the disturbances produced by ionization as well as of the conditions encouraging or discouraging breakage, restitution, and reattachment of broken ends. KAUFMANN (1941), recognizing this, has instituted investigations with infrared designed to alter if possible the irradiated sperm of Drosophila during the time between radiation and fertilization. The results of KAUFMANN'S study, however, have not as yet been published.

A series of preliminary experiments have been carried out to determine the effect of short-wave ultraviolet (2537 Å) on X-rayed pollen tube chromosomes of Tradescantia. It will be recognized that the more precise the tool used in such a study, the more informative will be the data obtained. Ultraviolet, in this respect, possesses several unique advantages. In addition to the fact that it can be successfully combined with X-rays by employing the pollen tube technique (SWANSON 1940a), any effect that it might have on X-ray breakage

[1] Journal Article No. 649 (n.s.) from the MICHIGAN AGRICULTURAL EXPERIMENT STATION.

or reattachment would extend the possibility of securing an additional spectral curve (cf. STADLER 1939), leading perhaps to the identification of chromosomal constituents intimately concerned with such phenomena. Ultraviolet also yields data which may be subjected to strict quantitative analysis.

The data presented in this paper indicate that short-wave ultraviolet, applied within certain temporal limits relative to X-raying, can lead to a considerable inhibition of X-ray induced breaks, the degree of inhibition being dependent upon the ultraviolet dosage and the time of application. The fact that ultraviolet, like X-rays, produces chromosomal aberrations introduces certain complications, but since the changes induced by ultraviolet are for the most part of a different nature from those induced by X-rays, the distinction between them is fairly readily made and may in turn be used to advantage in further distinguishing between the effect of these two agents.

MATERIALS AND METHODS

A clonal line of *Tradescantia paludosa* Anders. & Woodson was used for this study. The data were derived from the irradiation of pollen tube chromosomes, using a technique previously described (SWANSON 1940a). The X-ray dosage was given at a rate of 123.6 r/min. from a Coolidge tube operating at 60 kv. The pollen tubes were held at 10 inches from the target. The ultraviolet source was a low pressure arc (Hanovia SC-2537), operating at 7500 volts and 120 milliamperes and emitting the greater part of its radiation at the 2537 mercury line. All radiations were carried out with the slides on which the pollen tubes were growing in a moist chamber fitted with an inch-deep water cell of fused quartz as a window. This permitted prolonged exposures without danger of desiccating the pollen tubes. Unfortunately no device was available for intensity measurements of the ultraviolet so that the dose can be given only in seconds. The data were derived from two experiments carried out during the summer and fall of 1942, each involving approximately the same number of chromosomes. Each figure in the total chromosome columns (tables 1 and 2) represents, on an average, an analysis of five slides. Since the results of the two experiments were quite similar in most respects, the data were lumped.

It should be emphasized here that ultraviolet-induced breaks can involve but a single chromatid at any one locus, with the expression of the break ranging from a partial lesion of the chromatid to complete detachment of the fragment. For the most part, the fragments seem to be separated from the remainder of the chromosome by an achromatic lesion (see fig. B, C, D, E, SWANSON 1942). The technique employed does not permit a further study of the deletions, but the similarity of previous ultraviolet studies (SWANSON 1940b, 1942) with the cytogenetic results of Stadler and his co-workers (STADLER 1939, 1941) leaves little doubt but that they are true deletions. To insure uniformity in scoring, only those breaks showing a clear lesion extending through the entire diameter of the chromatid were classified as single deletions. This method was also followed in scoring X-ray breaks of a similar nature. Where the chromosomes were exposed to both types of radiation it was not possible to distinguish by vision between the X-ray-induced and the ultraviolet-induced single deletions.

OBSERVATIONS

The effect of ultraviolet pre-treatment

The effects of ultraviolet on the production of X-ray breaks may be determined by irradiations before, during, or after X-ray treatments. Since technical difficulties precluded an ultraviolet treatment during X-radiation, only pre- and post-treatments were attempted.

TABLE 1

Effect of ultraviolet pre-treatment on the production of x-ray breaks (%).

TREATMENT*	SINGLE DELETIONS		DOUBLE DELETIONS		TRANSLOCATIONS		TOTAL BREAKS		TOTAL CHR.
	OBSERVED	EXPECTED	OBSERVED	EXPECTED	OBSERVED	EXPECTED	OBSERVED	EXPECTED	
uv/7½"/1 hr.	.39	—	—	—	—	—	.39	—	3084
uv/15"/1 hr.	1.01	—	.08	—	—	—	1.09	—	2376
uv/30"/1 hr.	2.25	—	—	—	—	—	2.25	—	2934
uv/60"/1 hr.	3.05	—	—	—	—	—	3.05	—	3540
x/2'/2 hr.	3.51	—	1.05	—	1.89	—	6.45	—	5304
x/3'/2 hr.	4.70	—	1.94	—	3.71	—	10.35	—	2532
uv/7½"/1 hr.+x/2'/2 hr.	2.53	3.90	.80	1.05**	2.53	1.89	5.86	6.84	2526
uv/15"/1 hr.+x/2'/2 hr.	2.59	4.52	1.11	1.05	.74	1.89	4.44	7.54	2430
uv/30"/1 hr.+x/2'/2 hr.	1.73	5.76	.78	1.05	.17	1.89	2.68	8.70	2310
uv/60"/1 hr.+x/2'/2 hr.	3.01	6.56	.35	1.05	.35	1.89	3.72	9.50	2256
uv/30"/1 hr.+x/3'/2 hr.	2.50	6.95	.81	1.94	.94	3.71	4.25	12.60	2964
uv/60"/1 hr.+x/3'/2 hr.	3.13	7.75	.62	1.94	.00	3.71	3.75	13.40	1920

* The symbols under the "Treatment" column are to be interpreted as follows: for example, uv/7½"/1 hr.+x/2'/2 hr. means a 7½ sec. dose of ultraviolet at 1 hour after the pollen was sown followed by a 2 min. X-ray treatment at 2 hours after the pollen was sown.

** The double deletions observed in the uv data have not been calculated into the "expected" values.

Table 1 presents the data derived from a series of pre-treatments in which the ultraviolet dose was varied and the time of treatment was kept constant. The ultraviolet was given one hour after the pollen tubes had been placed on the agar medium and one hour before X-raying. As judged by the percentages of the total number of chromosome breaks, the data show that such a pre-treatment exerts a decided inhibitory effect on the realization of X-ray breaks, the observed percentage being clearly lower than that expected were the breaks cumulative. Since there is no apparent reason at this time for assuming that the X-rays exert an influence on the production of ultraviolet-induced breaks, it is quite possible that the reduction, or inhibitory effect, is entirely at the expense of the X-ray induced types.

The inhibitory effect is increased with an increase in the ultraviolet dosage. It cannot as yet be stated whether the amount of inhibition is proportional to the dosage of ultraviolet applied. Indeed there appears to be a saturation effect operative at the higher ultraviolet (30 and 60 sec.) doses, as indicated by a leveling off in the amount of reduction. This is further suggested by the results obtained when the X-ray dosage is increased to a 3-min. (370.8 r) exposure. With a 60-sec. ultraviolet pre-treatment, there is no appreciable increase in total breaks (3.75 percent) over that found at the 2-min. X-ray dose (3.72 percent).

By breaking down the data for the individual types of breaks into percentage figures, it is possible to gauge more exactly the degree to which each is affected by the ultraviolet pre-treatment. Both observed and expected percentages are given. The data show that the various types—single deletions, double deletions, and translocations—do not behave in like manner, suggesting a differential reactivity. The single deletions show a reduction at the lowest ultraviolet dose employed, and they become gradually reduced in number until at the 30- and 60-sec. doses, they are to be found in no greater frequency than that expected from the ultraviolet alone. The increase in single deletions at the 60-sec. dose can be largely attributed to the increase in ultraviolet dosage. The data seem to suggest, therefore, that the X-ray induced single deletions have been completely eliminated, but the inability to distinguish between ultraviolet-induced and X-ray-induced deletions leaves this somewhat in doubt.

The inhibition of translocations by the pre-treatment is more pronounced than that of the double deletions. From the data there appears to be a rise in translocations following the $7\frac{1}{2}$-sec. ultraviolet dose, but since this is largely due to an increase found in only one of the two experiments, it is of doubtful significance. A drop in percentage is noticeable as the dose is increased, with an apparent leveling off at the higher doses as the result, probably, of a saturation effect. Raising the X-ray dose to a 3-min. exposure did not materially increase the production of translocations when the ultraviolet (at the 30- and 60-sec. doses) was held constant. A 60-sec. dose followed by a 3-min. X-ray exposure resulted in no detectable translocations.

Double deletions are the least affected of the various aberrations. Not until the ultraviolet was increased to 30- and 60-sec. doses (with the 2-min. X-ray exposure) was an appreciable effect noticed. On the other hand, the lowered percentage found following a $7\frac{1}{2}$-sec. dose indicates that some effect may be produced even by small amounts of ultraviolet. At the 60-sec. doses, one-third of the expected double deletions are still produced, and no saturation effect was evident. An additional increment of ultraviolet would probably be necessary to completely eliminate the double deletions. It is significant to note that at the 60-sec. dose, the double deletions, for the most part, were of a different type than that usually induced by X-rays. The sister chromatids appeared to be broken at identical loci, but instead of uniting to form the customary U-shaped fragment and dicentric chromatid, the broken ends were held in place without union and remained simply as two single deletions. It appears, therefore, that the ultraviolet not only reduces the number of double deletions, but prevents those that are produced from uniting broken ends as generally occurs following X-radiation.

The effect of ultraviolet post-treatment

To test the effect of ultraviolet when applied after the X-ray treatment had been given, post-treatments were made immediately after X-raying, and then at one-half and one hour intervals. For the sake of comparison, the results of a pre-treatment given immediately prior to X-raying are included (table 2).

The ultraviolet dose was kept constant at 30-sec., but since the ultraviolet breakage rate varies with the time of prophase development (SWANSON 1942), it was necessary to determine the effect of the ultraviolet at the various time intervals used in the post-treatments. All the X-ray exposures in this experiment were of 2-min. duration.

TABLE 2

Effect of ultraviolet post-treatment on the production of X-ray breaks (%).

TREATMENT	SINGLE DELETIONS OB- SERVED	SINGLE DELETIONS EX- PECTED	DOUBLE DELETIONS OB- SERVED	DOUBLE DELETIONS EX- PECTED	TRANSLOCATIONS OB- SERVED	TRANSLOCATIONS EX- PECTED	TOTAL BREAKS OB- SERVED	TOTAL BREAKS EX- PECTED	TOTAL CHR.
uv/30″/2 hr.	3.17	—	—	—	—	—	3.17	—	1704
uv/30″/2½ hr.	2.99	—	—	—	—	—	2.99	—	2004
uv/30″/3 hr.	2.79	—	—	—	—	—	2.79	—	2436
x/2′/2 hr.	3.51	—	1.05	—	1.89	—	6.45	—	5304
uv/30″/2 hr.+x/2′/2 hr.	2.25	6.66	1.46	1.05	.45	1.89	4.17	9.62	1776
x/2′/2 hr.+uv/30″/2 hr.	3.29	6.66	1.17	1.05	.00	1.89	4.46	9.62	2556
x/2′/2 hr.+uv/30″/2½ hr.	2.25	6.49	1.02	1.05	1.43	1.89	4.70	9.44	1956
x/2′/2 hr.+uv/30″/3 hr.	3.72	6.29	1.68	1.05	2.32	1.89	7.72	9.24	1922

The data in the total break column indicate that short-wave ultraviolet can materially inhibit the degree of X-ray breakage even when applied as a post-treatment. This indicates that not all of the X-ray breaks and rearrangements are immediately realized, a conclusion that has been previously drawn by other investigators using other methods (MULLER 1940; KAUFMANN 1941; SAX 1930). Double deletions appear to be little affected by a post-treatment of ultraviolet. If anything, there is a slight increase, although the significance of this needs further verification. Translocations are markedly reduced when the ultraviolet either just precedes or follows the X-raying, but if a one-half hour interval is allowed between the two treatments, the reduction is slight. These data are in keeping with the conclusion drawn by SAX (1940) that most reattachments in the microspores of Tradescantia are completed within an hour's time.

Single deletions, on the other hand, show a decrease throughout the entire range of post-treatments. The inhibition appeared to be less marked after one hour had intervened between the X-raying and the ultraviolet treatment. The explanation for this continued reduction is not readily apparent, but it may be that the ultraviolet leads to an increased rate of restitution even if applied long after the X-raying.

The lack of effect of the ultraviolet on double deletions seems to indicate that this type of breakage is realized at the time of radiation, having no appreciable delay between breakage and reattachment. The data in table 2 also suggest that the inhibitory action of ultraviolet is not immediately effective. Pre-treatments given an hour before X-radiation are effective in reducing the double deletions, but if given just prior to the X-rays, no reduction is evidenced. It is possible that certain gelation phenomena may be involved in this reaction, with completion of the reaction requiring a certain time interval.

DISCUSSION

The data presented show that, within limits, pre- or post-treatment with short-wave ultraviolet leads to a reduction in the number of chromosome breaks realized from a given X-ray dose. The types of breaks react variously to these treatments, but by varying the time of treatment and the dosage, all can be materially inhibited. The change produced in the chromosome or the cytoplasm by the ultraviolet treatment is at present obscure, but it appears most likely that some constituent of the chromosome undergoes an alteration on absorption of the ultraviolet. Previously published data (SWANSON 1942) suggest that this constituent might be the chromosome matrix. When subjected to short-wave radiation (2537 Å) of sufficient dosage, the matrix tends to become quite conspicuous, the chromosomes appear to be contracted more than usual, and they fail to separate readily into two distinct and free chromatids. With longer wave lengths (2967 and 3022 Å) this does not occur even though the dosage, in ergs, be increased 20-fold. (It should be pointed out, however, that in this study the ultraviolet doses were not sufficiently heavy to produce a visible change in the matrix.)

The differential inhibition of the single deletions and translocations on the one hand and the double deletions on the other lends some support to this matrical conception. The ease with which the single deletions and translocations are inhibited suggests that the ultraviolet prevents single breaks from entering into new associations by interfering with the normal behavior of the matrix, possibly by increased gelation of the matrical substance which makes it more resistant to breakage. It has been emphasized before that breaks produced by ultraviolet do not as a rule enter into new rearrangements, nor do the single deletions become dislocated from the remainder of the chromosome but remain attached and in position, separated from the rest of the chromosome only by an achromatic lesion. This behavior again in all probability relates to the matrix, for, as MULLER (1940) points out, the ultraviolet cannot disrupt the matrix to produce a "thoroughgoing break," whereas an X-ray break with its subsequent reattachment must involve both matrix and chromonema. Double deletions do not appear to be so affected. Despite the ultraviolet treatment, these breaks are regularly, although sometimes less frequently, produced, and they involve both matrix and chromonema. Only at the higher pre-treatment doses do the double deletions not show the characteristic U-shaped fragment and dicentric chromatid, but show instead parallel chromatids each having a deleted portion with the breaks at identical loci. The failure of the fragments to unite to form a U-shaped fragment very likely may be attributed to interference by an altered matrix. The fact that double deletions may be formed when single deletions and translocations are readily inhibited suggests that they might be formed first within the fused matrices of the two chromatids. The gap in the broken chromonemata will form a weakened locus which will lead to a disruption of the matrix. This is supported to a certain extent by observations which show that while U-shaped fragments are produced after, say, a 30-sec. ultraviolet pre-treatment, they are usually attached to the remainder of the chromosome by a transparent connection which is undoubtedly

derived from, or is a portion of, the matrix. Where X-ray breaks are prevented by the matrix from entering new associations with other similar breaks, they probably undergo restitution, thus reducing the number of expected breaks visible at metaphase.

In connection with the assumption of a matrical control of chromosome breakage, it is of interest to consider two groups of seemingly conflicting observations. Visual observations of anaphase chromosomes leave little doubt that they possess a double structure—that is, the chromonema is split into two distinct chromatids. Yet, on the other hand, X-ray data (SAX 1940, and others) show that the chromosome during the resting stage behaves as a single unit in breakage and reunion. One is therefore forced to conclude that the subdivision of the chromosome does not determine the type of breakage and reunion which will result from X-radiation. The only remaining chromosomal structure of which we are aware is the matrix. Can the behavior of the matrix, whose structure and function are as yet but poorly understood, adequately account for chromosome behavior during and after radiation? Obviously our present knowledge does not permit of generalizations, but if it is assumed that the matrix remains undivided until prophase (apparently it is in anaphase) and that during the resting stage it maintains the unity of the chromosome as a single whole, then X-radiation would produce only chromosome, or 1-thread, breaks up until the time of matrix division, after which only chromatid, or two-thread, breaks would be produced. This transition period occurs at about 30 hours before metaphase (SAX 1940) in Tradescantia microspores.

That the ultraviolet post-treatment leads to increased restitution is suggested by the data in table 2. The single deletions still show considerable reduction even when the post-treatment is given an hour after X-radiation. Since the double deletions seem to be quickly realized and the translocations are completed within an hour after X-raying, it appears reasonable to assume that the single deletions are also realized within this same time interval. That the observed percentage is much below that expected, however, indicates that restitution has taken place after the ultraviolet has been given. MCCLINTOCK (1942) has beautifully demonstrated in maize, as have MULLER (1940) and KAUFMANN (1941) in Drosophila, that broken ends, or "potential breaks," are capable of remaining "open" for a considerable length of time. Restitution must also be possible under the same conditions, and in the pollen tubes of Tradescantia the ultraviolet appears to hasten this type of union.

In connection with the observations made by STADLER (1939, 1941), MULLER (1940), and SWANSON (1940b, 1942) to the effect that the ultraviolet breaks are qualitatively different from those produced by X-rays, it is significant to note that the single deletions produced by these two agents are not cumulative, even though each agent by itself can induce this type of break. Were they produced by the same mechanism, or were the broken ends similar, the single deletions should be cumulative. Since they are not, there is good reason, therefore, for believing that, despite their similar appearance, they are produced by different mechanisms, and that the manner of production determines the stability of the broken ends (cf. MCCLINTOCK 1941).

SUMMARY

A study of the effect of short wave length ultraviolet (2537 Å) on the production of X-ray breaks in the pollen tube chromosomes of Tradescantia led to the following conclusions:

Pre-treatment one hour before X-raying leads to an inhibition of all types of visible X-ray breaks. Single deletions and translocations are more affected than are double deletions.

The degree of inhibition depends upon the dosage of ultraviolet used, although the exact proportionality is not known.

Post-treatment has no inhibitory effect on double deletions regardless of time of application. Translocations show considerable inhibition if the post-treatment is given immediately after X-radiation, slight inhibition if given one-half hour later, no inhibition if given one hour later. Single deletions are inhibited even after the one hour post-treatment, suggesting that the ultraviolet facilitates restitution.

It is suggested that the action of ultraviolet is not immediate, but that a certain time interval is required before it becomes effective. A further suggestion is made that the inhibition caused by the ultraviolet results from an effect produced on the chromosome matrix which leads to its greater resistance to X-ray breakage.

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