DIFFERENTIAL SENSITIVITY OF PROPHASE POLLEN TUBE CHROMOSOMES TO X-RAYS AND ULTRAVIOLET RADIATION*

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PLATE 4

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INTRODUCTION

The sensitivity of a cell to radiation depends in a large measure on the sensitivity of its nucleus (9). The sensitivity of the nucleus in turn varies with the stage of division and the rapidity of division (2, 3, 8, 12, 15, 17, 23, 25). The data of the various workers are not entirely in agreement, most stages of division, with the exception of interphase, being reported as the most sensitive or the most resistant. This disagreement stems in part from the variety of organisms used and the type of division (mitosis or meiosis) tested, and in part to two factors which can be obviated through the use of suitable techniques. These factors are the lack of a specific criterion for measuring sensitivity, and the difficulty of determining exactly the stage of division at which radiation is applied.

Two techniques, the pollen tube technique (21) in plants, and tissue culturing (4) in animals, are available which can remove most of the difficulties encountered in a problem of this sort. The former technique has been employed in this study. Because of the regularity of nuclear development in the pollen tubes, particularly in the 1 to 4 hour period after germination in *Tradescantia*, it is possible to ascertain with a good deal of accuracy the varying sensitivity of the chromosomes (as regards both primary and secondary effects) as it is correlated with changes in the developing nucleus. At the same time, the technique renders feasible a further comparison of the effects of x-rays and ultraviolet radiation on mitotic chromosomes, with chromosome breakage employed as the criterion of sensitivity.

Material and Methods

The clonal line of *Tradescantia paludosa* Anders. and Woodson used in this study was the same as that used in other x-ray and ultraviolet studies (23). The slides were prepared according to the pollen tube technique (21), with enough colchicine, replacing acenaphthene, dissolved in the sugar-agar-gelatine medium to give an 0.001 per cent solution. The colchicine replacement is advisable because of its ready and cer-

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tain action, and because it facilitates the chromosome analysis by spreading them widely in the pollen tube. Fixation was carried out approximately 24 hours after the pollen was sown. Since both x-rays and ultraviolet tend to impede prophase development, the greater the dose given, the longer the pollen tubes were allowed to grow before fixation. In order to keep the agar from drying out during the exposures to radiation, the slides, during exposure, were kept in a moist chamber having a water-filled cell of fused quartz as a window.

X-ray treatment was delivered by a Coolidge tube (60 kv., 10 ma.), with the pollen tubes 10 inches from the center of the target. The dose was 123.6 r/minute, measured by a Victorean dosimeter.

TABLE 1
Effects of Ultraviolet (2537 Å) at Successive Prophase Stages. (Taken in Part from Swanson (23)).

Time	Total chromosomes	Deletions	Total breaks
<i>hrs.</i>			<i>per cent</i>
0	1368	20	2.52
1	1708	54	3.16
2	1728	92	5.32
3	1404	57	4.06
4	1020	26	2.55
5	1134	13	1.14
9	1236	7	0.56
11	576	2	0.35
13	1002	2	0.20
15	1470	3	0.21

For the ultraviolet treatments, two low pressure mercury arcs, both of the Hanovia SC-2537 type, were used. Both yielded approximately 85 per cent of their radiation at wave length 2537, while the greater portion of the remainder was above the genetically effective region of the spectrum. Most of the ultraviolet data (Table I) were collected while the writer was at the University of Missouri (23, Table 6), and the light source employed operated at 5000 volts. At these laboratories, the data at the 0 and 1 hour stages, as well as checks on other stages, were obtained from a similar light source but one operating at 7500 volts. Comparable doses were utilized to permit the combining of the two sets of data.

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OBSERVATIONS

Differential Sensitivity to Ultraviolet

As demonstrated in previous experiments (22, 23) ultraviolet can produce, in the generative nucleus, only chromatid deletions. It is entirely possible that these deletions, through a delayed illegitimate fusion, may result in chromatid translocations at some later stage in division than metaphase, but up

until that time, no evidence has been forthcoming which would indicate otherwise. That this is so results probably from the fact that broken ends induced by ultraviolet are more stable than those arising spontaneously or those induced by the more ionizing types of radiation (11), although ultraviolet-induced translocations have been reported (19, 20).

The changing rate of deletions produced in the prophase stages of the generative nucleus indicates that secondary changes in the chromosomes, arising progressively in prophase, exert a profound influence on the rate of breakage. From the 2 hour stage on, a continuous decline obtains; the chromosomes become inherently more resistant as prophase advances. At 11 hours after the pollen grains are sown, there is no appreciable increase over the spontaneous rate. No *visible* deletions therefore are produced at these later stages by the incident energy. The technique unfortunately does not permit a study of the next cell division so that it cannot be stated with certainty that no breaks of any kind are produced at late prophase which might be realized at later divisions.

The 0 and 1 hour stages show a decreased rate of breakage as compared to the 2 hour stage. It would thus appear that the generative nucleus in its resting stage and earliest prophase is less sensitive to the effects of ultraviolet than are the later stages. Two factors, however, are apt to prove these data misleading. *Tradescantia* pollen has a tendency to clump at anthesis, thus rendering it difficult to dust it onto a slide in an even monolayer. There will be some shielding by the top pollen grains, thus materially reducing the amount of incident energy reaching the lowermost nuclei. Also, and this is very probably the more important factor of the two, the generative nucleus does not usually emerge from the pollen grain to pass into the tube until after the 1 hour period, necessitating thereby radiation through the wall of the grain. Uber (24) has demonstrated that while the transmission curve for pollen walls in maize has a maximum around 2500 Å, the actual transmission at this wave length is only 30 per cent. Although nothing can be said at present concerning the transmission by the pollen wall in *Tradescantia*, it is reasonable to assume that since it is heavily pigmented it would considerably reduce the amount of energy reaching the generative nucleus. It seems likely therefore that the data do not give a true picture of the sensitivity at the 0 and 1 hour stages, and it is suspected, though not proved, that the resting stage is more sensitive, so far as chromosome breakage is concerned, than are the prophase stages.

When the nucleus has passed into the tube transmission would be at its highest value for the nucleus is now free of the pollen wall, and the pollen contents are considerably diluted by the uptake of water. It should be pointed out, however, that Uber (24) has shown the pollen contents, exclusive of the wall, to have again a maximum at about 2500 Å, but with an actual transmission of only 20 per cent.

Differential Sensitivity to X-Rays

In comparing the effects of x-rays and ultraviolet on the pollen tube chromosomes (23), it was pointed out that during the 2 to 4 hour period after germination the effects of these two types of radiation were exactly opposite. The sensitivity to ultraviolet decreased while to x-rays it increased. This analysis has now been carried to a point where a comparison can be made for the whole prophase stage (Tables II and III), and it can now be stated that the differential reaction found at the 2 to 4 period is absent at later stages, both ultraviolet and x-rays showing a continued drop in breakage rate as prophase approaches metaphase.

TABLE II
Effects of X-rays at Successive Prophase Stages. Exposure: 3 Min. Dose: 370 r.

Time	Total chromosomes	Single* deletions	Double* deletions	Translocations*	Total breaks
<i>hrs.</i>					<i>per cent</i>
0	2400	8	20	12	2.16
1	1596	68	46	18	9.46
2	2532	119	49	47	10.35
3	2430	123	30	57	10.98
4	2136	135	28	54	12.72
5	1800	102	18	18	8.66
6	1872	56	0	24	5.55
7	1290	10	5	0	1.16
8	1872	6	6	0	0.62
10	1224	3	0	0	0.24
15	786	1	0	1	0.38
Control	2520	3	1	1	0.23

* Single deletion

Double deletion

Translocation

Taking the per cent of total breaks as an indication of the degree of sensitivity (Table II), the 4 hour period represents the point of maximum sensitivity. A gradual decline occurs at later stages until at the 10 hour period the breakage rate does not exceed that produced spontaneously. The later stages of prophase thus respond identically to both types of radiation.

Table III presents the various types of x-ray breaks in terms of per cent. Single deletions and translocations follow a similar trend, with their maxima at the 4 hour period. The close relationship of single deletions to translocations suggests that the former contribute to the production of the latter, thus lending additional support to the breakage-first hypothesis of Stadler. The double deletions reveal a rise at the 1 hour period, but the decline begins imme-

diately after. Sax (13) has shown that similar trends are to be found among x-ray aberrations in *Tradescantia* microspores. As Sax also emphasizes, the changes, with the exception of the sharp initial rise following germination, are gradual in nature and correspond to the gradual changes taking place in prophase.

TABLE III
*Effects (in Per Cent) of X-Rays at Successive Prophase Stages. Exposure: 3 Min.
Dose: 370 r*

Time	Total chromosomes	Single deletions	Double deletions	Translocations	Total breaks
<i>hrs.</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0	2400	0.333	.83	1.00	2.16
1	1596	4.26	2.88	2.25	9.46
2	2532	4.71	1.93	3.71	10.35
3	2430	5.06	1.23	4.69	10.98
4	2136	6.32	1.35	5.05	12.72
5	1800	5.66	1.00	2.00	8.66
6	1872	3.00	0.00	2.55	5.55
7	1290	0.77	0.38	0.00	1.16
8	1872	0.32	0.32	0.00	0.62
10	1224	0.24	0.00	0.00	0.24
15	786	0.13	0.00	0.25	0.38

The Correlation of Sensitivity with Prophase Behavior

In attempting the interpretation of any biological mechanism, it is desirable, if not essential, to seek relationships between form or structure on one hand and behavior on the other. When a relationship exists, and both are variable, then their variabilities should likewise show correlation. This is true for chromosome breakage, for, as has been pointed out (13, 15, and others), the mechanism of breakage through radiation is conditioned to a considerable extent by the structure and behavior of the chromosomes at the time of radiation or shortly thereafter. This can be clearly demonstrated in the generative nucleus because of its readily traceable progressive development, particularly in the early prophase stages where changes in sensitivity vary greatly. In later stages, however, there is an overlapping of stages which makes too close a comparison inaccurate and undesirable.

The generative nucleus in the pollen grain before germination (0 hour period) is elongated, highly chromatic, and compact (Fig. 1). There can be but a minimum of movement in such a condensed nucleus, and conditions are such that external stresses are not operative, thus favoring the *status quo* of the chromosomes. On the "direct hit" theory of Sax (14) this would signify a high rate of reunion; if x-rays produce potential breaks (10), then the realization of

breaks would be low. In either event, a low breakage rate obtains due to the absence of secondary factors. With the beginning of germination, the nucleus elongates and eventually passes into the pollen tube. Its length has by this time increased considerably (Fig. 2), and although there is some decrease in the width, the length increase is most likely made possible by an uptake of water, a phenomenon long known to accompany nuclear division. The initial 4-fold increase in x-ray breaks at the 1 hour period (Table II) is undoubtedly traceable to the stresses involved in the uptake of water, with the subsequent opening up of the relic coils as well as in the elongation of the nucleus and its movement as a whole in the tube. Although the individual chromosomes are distinct enough at the 1 hour stage there is no indication of the presence of a new somatic coil. This coil, however, is well established by the 4 hour period (Fig. 3), and other observations show that spiralization begins at about the 2 hour stage. Since the chromatid, and not the chromosome, is the unit of coiling, and is independent, to a certain degree, of its sister chromatid, it seems reasonable to assume on *a priori* grounds that a reduction would occur in number of double deletions while an increase would be found in the single deletions. Since the single deletions probably contribute to the translocations, a rise would be expected in the latter. This is what is found. The period of greatest activity (loss of relational and relic coils, initiation of somatic coils) coincides with the period of greatest sensitivity. Coiling during mid and late prophase (Figs. 4 to 6) is a more leisurely affair, and movement is restricted. Sax (13) has stated that double deletions can be produced by x-ray hits in chromosomes that are visibly split. In the generative nucleus, the split is first evident at the 2 hour stage, and although at this stage the per cent of double deletions is low when compared to other types of breaks, they are nevertheless produced in appreciable numbers, thus bearing out Sax's contention that a single hit can traverse the distance involved in breaking a double-stranded thread.

At the later stages of prophase, other factors must exert an influence on chromosome breakage, otherwise it is difficult to explain the absence of even single deletions. What these factors are can only be conjectured at this time, but undoubtedly the development of the matrix and the nucleic acid accumulation (5, 6) both act as stabilizing influences.

DISCUSSION

The data presented reveal that while a difference exists in the reactivity of the chromosomes to ultraviolet and x-rays during early prophase, the later stages (most of the middle and all of the late prophase) show a gradually decreased sensitivity to both types of radiation. This, in part, is in reasonable agreement with the data of Sax and Swanson (15) on *Tradescantia* microspores. It is not, however, in agreement with the more recent data of Sax (13), also on microspores, in that a fall rather than a continued rise of single deletions and

translocations is found from mid prophase on. The disagreement can result from two factors: (1) a differential behavior of the microspore and pollen tube chromosomes to x-rays, or (2) a difference in the interpretation of prophase stages. The second factor is probably the more important of the two. Sax considers prophase to begin with the effective splitting of the chromosomes, a condition which may or may not be true since in the microspores there is no method of determining exactly the stage of division irradiated. Again, if, in the generative nucleus, this criterion were used then prophase would begin 2 days before anthesis, since at this time chromatid breaks can be obtained. On the other hand, prophase in the pollen grain and tube appears no different than in other mitotic cells with the one exception that the generative nucleus is compact and elongated rather than large and spherical.

The impression is gained, from development studies of the generative nucleus, that two factors determine the change in sensitivity to x-rays in the 1 to 4 hour period. These are the uptake of water which leads to an elongation of the nucleus, and hence leads to much chromosome movement, and the initiation and continuation of the spiralization cycle. Later stages find the spiralization tempo much reduced, and the chromatids widely separated, resulting in fewer breaks. It seems unlikely, however, that the inability of x-rays, at the dosage used, to produce breaks of any detectable kind in late prophase can be adequately explained by the above factors unless it is assumed that breaks are produced but are not realized until the next cell division. Unfortunately this technique does not permit such an analysis.

That the activity of the chromosomes in prophase is inadequate to explain the decreasing sensitivity to ultraviolet has been emphasized (23). Also McClintock (11) has suggested, from genetical data, that the ultraviolet breaks are relatively stable, indicating that not only are the breaks not visibly realized but that in all likelihood they are not being produced at all. To state it otherwise, the diminishing rate is not due to an increasing rate of reunion. It becomes increasingly evident therefore that changes inherent in the structure of the chromosome condition its sensitivity to ultraviolet.

Since the efficacy of ultraviolet results from a selective absorption dependent upon the local properties of the irradiated substances, it is essential that the nature of these inherent changes be known if the mechanism of chromosome breakage is to be understood. While the chemical nature of the chromosome, and the alterations in its molecular structure which it undergoes while in division, are as yet but partially comprehended, there are three tentative aspects of the problem which, in relation to chromosome breakage, bear further investigation. These are (1) the changes in nucleic acid attachment, (2) the behavior and development of the matrix, and (3) further splitting of the chromosome. Their relative importance at this time is conjectural.

The division cycle of a nucleus is accompanied by a cycle of attachment

and detachment of nucleic acids to and from the protein framework of the chromosome (5, 6). Ultraviolet absorption techniques as well as staining methods show the maximum attachment to occur at metaphase, and the maximum detachment at interphase. Signer, Caspersson, and Hammersten (18) have further demonstrated that the molecules of nucleic acid polymerize in long chains which parallel the longitudinal axes of the backbone proteins with a spacing nearly identical with that of the polypeptides comprising the proteins (1). This suggests that the nucleic acids, as they accumulate, act as a protecting sheath to the encased proteins, thus preventing their dissociation by the action of ultraviolet. Since the accumulation continues up to metaphase, so the resistance will similarly increase. This may serve to explain the approximation of Stadler's (19) ultraviolet deficiency data in maize to a nucleic acid absorption curve. Additional support has been furnished by Greenstein, Jenrette, and Hollaender (7), who show that the action of ultraviolet on sodium thymonucleate is one of depolymerization. It is thus possible that depolymerization of the nucleic acids attached to the proteins of the chromosome is a necessary step before breakage can occur. Whether an explanation of the failure of x-rays to produce fewer and fewer breaks in late prophase (Tables II and III) is to be sought in a mechanism of this sort cannot at present be stated, although in view of Bozeman's (2) recent data, this seems unlikely.

The rôle of the matrix in chromosome breakage is problematical in view of our limited knowledge of the physical features and function of this structure. Metz and Bozeman (12) consider x-rays to affect the viscosity of the matrix. There is some evidence (23) that the matrix increases in dimension as the division cycle approaches metaphase, acting thus as a stabilizing factor in maintaining the integrity of the chromosome. Schultz (16) has demonstrated the presence, in *Drosophila* mitotic chromosomes, of a matrix of a complex protein bound to the chromosome proper by ribose nucleic acid linkages. Although the pattern of chromosome-matrix relationship remains obscure for the time being, the mere presence of this structure and the fact that a chromosome break must necessarily involve the matrix as well as the chromosome proper, emphasizes that it must be taken cognizance of in any consideration of the mechanism of x-ray or ultraviolet breakage.

A third factor which may exert an influence on the rate of ultraviolet breaks is the number of subdivisions in the chromosome at the time of irradiation. Sufficient evidence has accumulated from many varied sources to state safely that the interphase chromosome is double regardless of its reaction to x-rays. This doubleness can be best seen in anaphase, so division into half-chromatids must have taken place at metaphase or earlier. That half-chromatids are present in prophase is indicated by this study. In the first place, numerous breaks are found which extend only a part way across the diameter of the chromatid, suggesting that only one of the two half-chromatids had suffered

breakage. Also, one unequivocal and several less certain instances of half-chromatid translocations have been detected at the 2 hour stage following x-radiation. In the single clear case, the half-chromatids at the point of breakage could be traced with ease and clarity. If these observations are correct, and it is difficult to conclude otherwise, then two points become clear: first, that since ultraviolet is a dissociation agent, with a limited sphere of action, it is to be expected that most of the breaks produced would involve half-chromatids, and would therefore not be detected until the following cell division, and second, that ultraviolet can break a double-stranded thread, implying that other factors than the mere "hit," or absorption, are necessary for the realization of a break in the chromosome. This latter implication is supported by Stadler's (19) data on endosperm deficiencies when he shows that although the majority of ultraviolet deficiencies are fractional, some of them are entire, involving in this manner both strands of a split chromosome.

From the above discussion it is apparent that the explanations presented in interpreting the ultraviolet data are to be regarded as suggestive only, and must await further study. Their validity will be tested as our knowledge of the chemistry of the chromosome and the chemistry of radiation increases.

SUMMARY

1. Through use of the pollen tube technique it has been possible to study the sensitivity of prophase stages to x-rays and ultraviolet, and to correlate the varying sensitivity with changes in the generative nucleus of *Tradescantia*.
2. Sensitivity to ultraviolet decreases from the 2 hour stage until at 11 hours after germination there is no further production of breaks. The 0 and 1 hour stages show a decreased sensitivity over the 2 hour stage but it has been suggested that this is not due to a decreased sensitivity but to shielding by the pollen wall.
3. Sensitivity to x-rays rises to a peak at the 4 hour stage, but then subsides until no breaks are realized (at a dose of 370.8 r) after the 10 hour stage. In this respect the effects of x-rays and ultraviolet are similar. Each type of x-ray break shows its own individual trend.
4. Correlation of x-ray breaks with changes in the generative nucleus indicates that the important events determining the sensitivity of the chromosomes to breakage are the uptake of water at the time of germination and the movement involved in spiralization. The total absence of breaks after the 11 hour stage is not understood.
5. The changing sensitivity to ultraviolet may depend on any one or all of three factors: (a) the nucleic acid cycle, (b) changes in the matrix, and (c) the number of subdivisions in the chromosome. These are discussed although their relative importance is not known.

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EXPLANATION OF PLATE 4

Figs. 1 to 6. Successive prophase stages in the generative nucleus.

Fig. 1. Mature pollen grain prior to germination; generative nucleus above, tube nucleus below.

Fig. 2. 1 hour stage of generative nucleus.

Fig. 3. 4 hour stage.

Fig. 4. 6 hour stage.

Fig. 5. 8 hour stage.

Fig. 6. 15 hour stage.

(Swanson: Sensitivity of prophase pollen tube chromosomes)