tion that follows from this interpretation of the mechanism of nitrite mutation and of reversion by d-lysine. An important, if not most important rôle is here assigned to d-lysine in the process of differentiation and reproduction, and concomitantly as a possible basis for strain differences in fruitfulness and certain growth characteristics. Progressive increase in sterility in successive transfers of fungi may, in some instances, have its origin in this process, and may be found possible of counteraction in the presence of excess calcium carbonate by means of d-lysine or of ammonium salt and thiosulfate.

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A COMPARISON OF CHROMOSOMAL ABERRATIONS INDUCED BY X-RAY AND ULTRA-VIOLET RADIATION

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The investigations of Stadler and Sprague[1] on maize, following that of earlier workers,[2,3] have confirmed and furthered our knowledge as to the genetical effects of ultra-violet radiation. They showed that like x-rays, ultra-violet radiation could materially increase the mutation rate, but that on the contrary it differed from x-rays in that no increase in the frequency of translocations was found. Besides point mutations affecting seed and seedling characters, numerous entire and fractional endosperm deficiencies were found, being similar in nature, although differing in relative frequency, from those produced by x-rays. Later Singleton,[4] and Singleton and Clark,[5] showed that plants with defective pollen segregations frequently revealed deficiencies that were chromosomal in nature and not simply gene mutations. These deficiencies were cytologically demonstrable at pachytene, and invariably involved the terminal deletion of a chromosome segment. Muller and MacKensie[6] confirmed the results of Stadler and Sprague in so far as translocations were concerned.

The accumulated evidence to date therefore points to a qualitative difference existing between the effects of ultra-violet and x-ray radiation. In all previous ultra-violet experiments, however, examination of individuals for chromosomal changes was made only after many cell generations had intervened since the time of treatment. A direct comparison of the results

of ultra-violet and x-ray radiation, i.e., examination before the intervention of a cell generation between the time of irradiation and that of observations, might serve to settle the question of whether or not a truly qualitative difference does exist between the effects resulting from these two kinds of radiant energy. It is with this purpose in mind that the present study has been undertaken.

A direct x-ray analysis of chromosomal aberrations is a comparatively easy matter because the extreme penetrating power of these rays permits them to reach cells whose division cycle is fairly accurately known and timed,[7] facilitating in this manner the collection of an extensive amount of data before the elimination of non-viable chromosome alterations. A direct analysis of the effect of ultra-violet treatment has not been too successfully carried out to date because of the drastic absorption of the ultra-violet rays by overlying tissues. However, with the development of

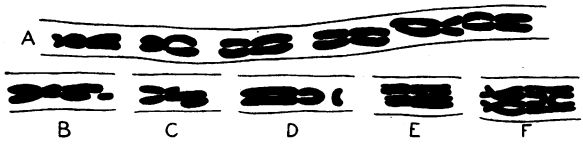

FIGURE 1

Camera lucida drawings of chromosomes in pollen tubes of Tradescantia. Ca. 2000 ×. *A*. Six normal chromosomes in pollen tube. Illustrates manner in which they pass down the tube following acenaphthene treatment. *B* and *C*. Chromatid deletions induced by ultra-violet radiation. *D*. Chromatid dicentric induced by x-rays. *E* and *F*. Chromatid exchanges induced by x-rays.

methods for growing pollen tubes on cultural media,[8] a singularly simple yet effective means is available for carrying out a direct cytological analysis of any chromosomal aberrations or rearrangements induced by ultra-violet radiation (figure 1*A*). In this manner, the generative nucleus can be irradiated in the pollen tube, absorption, therefore, being at a minimum.

The plants used in this study were from a clonal line of a diploid species of Tradescantia. The source of the x-rays was a Coolidge tube equipped with a tungsten target. The source of the ultra-violet radiation was a Hanovia mercury arc lamp operating at 4 amperes and 110 volts d. c. The light was unfiltered, and the treating distance was 10 cm. The heating effect was reduced by the use of an electric fan.

X-Ray Effects on Microspores.—An extensive analysis of x-ray induced chromosomal aberrations in Tradescantia microspores has been carried out by Sax[7] and others. These induced changes fall into two readily distinguishable classes, their nature depending upon the conditions of the

chromosomes at the time of irradiation. Chromosome breaks result from treatment given when the chromosomes are in the form of single threads; chromatid breaks are induced at prophase after the chromosomes have become functionally split into two sister chromatids.

Chromosome breaks are of several kinds, depending upon the number of effectively broken chromosomes concerned. A single break generally involves the deletion of a portion of an arm, the deleted segment lying in the cytoplasm as an acentric fragment. Dicentric chromosomes, ring chromosomes and exchanges between non-homologous chromosomes are also frequently observed.

Chromatid breaks can likewise be classified as to their one-hit or two-hit nature. Single breaks may include one or both of the sister chromatids. If one is broken, this is visible as a shortened chromatid accompanied by a deleted fragment; if both are broken, lateral fusions between the broken ends of sister chromatids give a dicentric chromatid and a U-shaped fragment. Independent hits in two adjacent chromosomes may result in a chromatid exchange; if in the same chromosome, a ring will be produced.

X-Ray Treatment of Dry Pollen Grains.—Open flowers were given doses of 200 r, and the pollen grains germinated on a cultural medium. The data are given in table 1.

TABLE 1

X-RAY DATA ON POLLEN GRAINS IRRADIATED DRY. 200 R

Figures represent number of chromosomes examined

NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	CHROMATID EXCHANGES	DICENTRIC CHROMOSOMES	TOTAL	% BREAKS
979	4	15	2	2	1002	2.29

Chromosome changes induced at this stage of pollen maturity are very similar in nature to those resulting from irradiation of early prophase nuclei in the microspores. The presence of two dicentric chromosomes indicates that the chromosomes of the generative nucleus at the time of pollen maturity are not all effectively split into sister chromatids. Dicentric chromosomes are never found when microspores are irradiated in prophase, so that their presence in the pollen tube is a valid criterion of the singleness of some of the chromosomes.

X-Ray Treatment of Pollen Tubes.—Pollen tubes were subjected to x-rays at approximately two hours after germination. As will be seen later, this particular time for irradiation was chosen so that a strictly comparable set of data might be obtained to test for the presence or absence of a qualitative difference between the effects of x-ray and ultra-violet radiation on chromosome breakage. The dosage was 240 r. The pollen tubes, however, were irradiated while still in the glass moist chamber in which

they were growing so that the actual dosage reaching the nuceli was somewhat lower due to partial absorption of the rays by the glass. The data are given in table 2.

TABLE 2

X-RAY DATA ON POLLEN TUBES. 240 R

NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	CHROMATID EXCHANGES	TOTAL	% BREAKS
946	29	22	5	1002	5.58

Under this treatment, chromatid deletions are more numerous than chromatid breaks (figure 1*D*), although both are undoubtedly the result of single quantum "hits." The high percentage of deletions is considerably above that found in x-rayed microspores whether rayed in prophase or resting stage, Sax[7] reporting that only five per cent of the aberrations induced at resting stage are of this type as opposed to over fifty percentage here. It is quite probable that an explanation may be sought in the spatial relationships of the sister chromatids at this time. The threads are without doubt farther apart when the generative nucleus has passed out into the tube than when it lies passively in the ungerminated pollen grain. Also a considerable tension is exerted on the chromosomes as they pass down the tube. This may be deduced from the fact that frequently the nucleus becomes separated into two or more independent groups of chromosomes, and also from the fact that occasional chromosomes are stretched almost to a breaking point as they move downward. This tension is probably set up by rapid protoplasmic streaming. Internal movements in the individual chromosomes as the result of prophase contraction may also be another factor in keeping broken ends from refusing into their original positions once they have become separated from each other. If this be the case, we might expect that the number of deletions found in irradiated pollen tubes reflects more nearly the actual percentage of breaks induced by this treatment than does the observed percentage of breaks in the microspores, where, according to Sax,[7] the frequency of actual breaks undoubtedly is much greater than the observed frequency, the majority of them reuniting back into their original positions.

In addition, chromatid exchanges (figure 1*E*, *F*) between non-homologous chromosomes are also present. No dicentric chromosomes were found. Evidently completion of the effective splitting had taken place by this time, and all aberrations could be classified as chromatid breaks.

Ultra-Violet Treatment of Pollen Tubes.—Pollen grains were germinated on slides and the generative nucleus irradiated two hours after sowing. Exposure was for 30 seconds. Longer exposures were found to be inadvisable because the film of medium soon becomes desiccated, inhibiting

the growth of the pollen tubes and finally causing death. Three different groups of slides were analyzed (table 3).

TABLE 4

ULTRA-VIOLET DATA ON POLLEN TUBES

30-second exposures at distance of 10 cm.

NORMAL	CHROMATID DELETIONS	CHROMATID DICENTRICS	TOTAL	% BREAKS
(*a*) 2155	23	0	2178	1.05
(*b*) 1273	11	0	1284	0.86
(*c*) 1565	11	2	1578	0.82
Con. 1587	2	1	1590	0.18

Experimental evidence reveals that the chromosomal aberrations induced by ultra-violet radiation are almost exclusively of the chromatid deletion type (figure 1*B*, *C*). So far as could be determined cytologically all deletions were terminal in nature, and of varying length, the breaks not being localized in any particular regions. All gradations from free fragments to achromatic lesions were found. However, only those breaks which unquestionably showed broken ends were considered in this study, although some of the achromatic lesions were undoubtedly true deletions which had not as yet become separated from the remainder of the chromosome. No lesions were found that extended across both chromatids at the same locus.

Only two chromatid dicentrics were observed in the ultra-violet treated material (figure 1*D*). Unlike the deletions, these aberrations involve breakage of both chromatids at the same locus, followed by lateral fusion to give the dicentric chromatid plus the U-shaped fragment. One of the two breaks lacked the usual fragment, and an examination of the entire length of the pollen tube and grain failed to reveal it lying in the cytoplasm. Evidently the above dicentric chromatid was the result of some previous microspore aberration which had been carried over into the generative nucleus. The only other chromatid break was similar to that found in the control, and probably was of spontaneous origin.

Discussion.—It has thus been possible to obtain a direct analysis of ultra-violet induced chromosomal aberrations, confirming in this manner the genetical results of Stadler and Sprague[1] and of Muller and MacKensie.[6] Cytologically observable deficiencies occur, but no increase in the percentage of translocations was found. The fact that a single effective ultra-violet "hit" can only break a single thread at any one locus while a single x-ray "hit" can break both threads seems best explained by assuming that the sphere of influence of an absorbed ultra-violet quantum is an area no greater in diameter than the width of a single chromatid, while that of an x-ray quantum is sufficiently wide in extent to include both. On the basis of energy values this becomes explicable, for as Good-

speed and Uber[9] point out, the energy of an ultra-violet quantum is from 4 to 6 electron-volts, whereas that of an x-ray quantum may range from 10,000 to 200,000 or more, the value increasing inversely as the wave-length.

The difference in the width of this so-called "sphere of influence" may perhaps be thought of as an expression of the difference in the physical behavior attending the absorption of these quanta. The curves obtained when breaks[7] or survival ratios[10, 11] are plotted against dosage imply the effectiveness of single quantum absorptions. It is now well established that the ionization created by x-rays is responsible for the chromosome breakage. Ultra-violet, on the other hand, because of the low energy values of a single quantum, cannot create a path of intense ionization. Instead, it derives its effectiveness from the fact that it can excite the absorbing molecules to higher energy states by affecting the electrons in only the outer orbits. Ultra-violet has, on this account, been frequently spoken of as "chemical rays." This ionization is therefore highly localized, and does not extend across the distance between the sister chromatids to cause a similar reaction at the same locus in the other thread.

This, however, does not explain the absence of translocations under ultra-violet treatment. The question arises as to whether or not chromosome breaks induced by ultra-violet radiation are capable of reuniting back into their original positions or into new associations. Sax[12] has shown that x-ray induced breaks can remain in an unstable condition and capable of re-fusion for as long as an hour after the time of irradiation. Does the absorption of an ultra-violet quantum leave a broken end in such a labile condition, or does the chemical action of these rays leave a satisfied bond at the broken end such that re-fusion is impossible? For the present this question must remain unanswered, but its solution might serve to explain the absence of gross chromosomal rearrangements with ultra-violet treatment under circumstances where comparable doses of x-ray, as judged from the frequency of endosperm deficiencies[1] and lethals,[6] produce an abundance of these types of aberrations.

Ultra-violet radiation of maize pollen[1] yields two kinds of endosperm deficiencies, entire and fractional, as judged by the absence of dominant endosperm characters. The fractional deficiencies generally consist of kernels showing 1/2 of the tissue recessive and the other 1/2 dominant, with about equal numbers of larger and smaller proportions. If we assume that the aberration induced by the treatment consists of the deletion of a segment of the chromosome arm when the chromosome is in the two-thread state, the production of fractionals becomes readily understandable. This is indicated by Stadler and Sprague's data which show that losses of *C* and *Wx* are usually associated, eliminating the possibility that these are simple point mutations. The first division of the endosperm-

fusion-nucleus, resulting from fertilization by such an altered sperm cell, would therefore give one cell with a normal, and one with a deleted, chromosome. The amount of endosperm derived from each of these cells would be approximately equal, giving kernels chiefly of the "1/2" class, but if the products of the two cells were unequal, as well might be the case since division in the endosperm is an irregular process, then equal numbers of larger and smaller proportions would be expected.

The production of entire endosperm deficiencies by ultra-violet treatment is not so easily explained. There are four possible ways by which this might be brought about: (1) ultra-violet induces breaks in both chromatids of a single chromosome arm by two independent absorptions, although on a random basis this seems very unlikely if all breaks are simple deletions; (2) a single "hit" can induce a chromatid break involving both chromatids at the same locus, a situation not yet demonstrated cytologically; (3) a single quantum might be absorbed in the region of the centromere where the thread is effectively single although the chromatids are split, giving a deletion for both of the chromatids of an arm; (4) if all of the chromosomes in the sperm nuclei or generative nucleus are not effectively split at the time of irradiation an opportunity is provided for a single "hit" to delete the entire end of a chromosome arm, thus giving an endosperm-fusion-nucleus deficient for that particular segment. At present this explanation seems to be the most satisfactory, and is not without some experimental evidence, for, as has been pointed out above, the appearance of dicentric chromosomes in pollen grains irradiated dry is good factual evidence for the presence of single threads at the time of irradiation. Furthermore, in maize, where division of the generative nucleus to give two sperm cells takes place in the grain instead of in the pollen tube as in Tradescantia, the possibility of having single threads in the sperm nuclei is obviously greater than in the generative nucleus of Tradescantia since no further division of the sperm nuclei takes place before fertilization.

X-ray induced endosperm deficiencies[1] are generally of the "entire" kind, while those induced by ultra-violet are largely fractionals. The experimental data obtained in this study are in good agreement with these conclusions since the chromatid deletions resulting from ultra-violet radiation would give genetically observable fractional deficiencies while the greater proportion of chromatid dicentrics induced by x-rays would result in entire endosperm deficiencies.

Summary.—X-ray radiation of the generative nucleus in Tradescantia pollen grains reveals that most of the chromosomes are effectively split into sister chromatids. A great proportion of the chromatid aberrations involve deletions of both chromatids of a chromosome at identical loci, thus confirming the genetic data in maize in respect to endosperm deficiencies. Irradiation of the generative nucleus in the pollen tube results

in only chromatid aberrations including a considerable number of simple chromatid deletions and occasional chromatid exchanges

Ultra-violet radiation of the generative nucleus in the pollen tube induces only simple chromatid deletions. The loss of only one of the two chromatids is in accord with the genetic observations that ultra-violet radiation produces primarily fractional endosperm deficiencies in maize. No configurations representing an interchange of chromatin between non-homologous chromosomes were found.

The qualitative difference between the types of breaks induced by x-ray and ultra-violet radiation is tentatively explained by assuming that the sphere of influence of a single x-ray quantum is much greater in area than that of a single ultra-violet quantum. The vast difference in energy values, and the difference in the physical behavior attendant to absorption of the respective quanta supply a possible physical and chemical basis for this variation in degree of effectiveness.

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CYTOLOGY AND DEVELOPMENT OF THE EMBRYOS OF X-RAYED ADULT DROSOPHILA MELANOGASTER

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In 1927 Muller[1] conclusively demonstrated that mutations could be induced in animals by means of x-rays. Exposure of the germ cells of *Drosophila melanogaster* to relatively heavy doses of x-rays resulted in the production of large numbers of mutations of different types, including recessive lethals, semi-lethals and visibles of various kinds. "In addition, it was also possible to obtain evidence in these experiments for the first time, of the occurrence of dominant lethal genetic changes, both in the X