GENETIC EFFECTS OF ULTRA-VIOLET RADIATION IN MAIZE. III. EFFECTS OF NEARLY MONOCHROMATIC λ 2537, AND COMPARISON OF EFFECTS OF X-RAY AND ULTRA-VIOLET TREATMENT

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A high voltage mercury discharge tube of fused quartz emits radiation consisting chiefly of λ 2537. A commercial tube of this sort (Hanovia (Sc-2537), operated at 5000 volts and 100 m. a., was used as a source of radiation in comparison with filtered radiations of the types described in the preceding paper.

The discharge tube treatments were applied under conditions similar to those used with the mercury arc. Exposures were made at the same distance and with the same filter cell, with 1 cm. of distilled water in place of the filters used with the mercury arc. Under these conditions about 83% of the total intensity is λ 2537. Other wave-lengths included are chiefly in the near ultra-violet and visible. Of the wave-lengths shorter than λ 3130, more than 97% of the total intensity is λ 2537. For the purposes of genetic experiments, therefore, this radiation is almost monochromatic λ 2537. The ratio of intensity of the radiation from the discharge tube and the arc (the water filter being used in both cases) was for λ 2537 approximately 1:1, for total radiation shorter than λ 3130 approximately 1:8.

In experiments with the discharge tube, the multiple recessive seed parent used was *A a C c wx wx su su pr pr y y*. The pollen stock carried the dominant alleles of all of these genes. It was therefore possible to detect one-half of the losses of *A* and *C* (as indistinguishable color losses) and all of the losses of *Wx*, *Su*, *Pr* and *Y*.

The maximum dose tolerated is considerably lower than the maximum tolerated dose of the filtered radiations reported above. Only the most approximate comparisons may be made in tolerance because of the variation with environmental conditions which has been mentioned. In treatments with the discharge tube, under favorable weather conditions and with excess pollen, well-filled ears are produced by the use of pollen irradiated for 30 seconds or less, but only partial seed setting occurs with pollen irradiated 60 seconds or more. A few seeds are produced by the use of pollen treated as long as 120 seconds. Under similar conditions, with filtered radiation *A* of the mercury arc (described in the preceding paper), well-filled ears are produced by the use of pollen irradiated 2 minutes and sometimes 4 minutes, and a few seeds are produced by pollen treated as

long as 16 minutes. The total intensity of ultra-violet radiation of wave-lengths shorter than λ 3130 is considerably higher in radiation *A* than in the discharge tube radiation, but about 90% of it is λ 2967 and λ 3022. When the mercury arc is used with the water filter, the maximum exposures tolerated are only about half as long as with the discharge tube. These observations indicate that the wave-lengths which must be chiefly responsible for the genetic effects of filtered radiations *A* and *B* (λ 2967 and λ 3022) have much less "killing" effect than λ 2537 and other short wave-lengths.

TABLE 1

COMPARATIVE EFFECT OF VARIOUS ULTRA-VIOLET RADIATIONS ON FREQUENCY OF ENDOSPERM DEFICIENCIES AND GERMLESS SEEDS

	DISCHARGE TUBE WATER FILTER	MERCURY ARC FILTER A	MERCURY ARC FILTER B	CONTROL
No. Seeds Examined	1199	695	525	578
Mean Exposure Period[1] (Sec.)	30.8	128.4	217.8	....
Deficiencies				
Entire (E)				
$\frac{A+C}{2}$	17	8	4	0
Pr	13	10	5	1
Su	10	7	4	0
Y	6	2	4	2
Fractional (F)				
$\frac{A+C}{2}$	28	23	8	2
Pr	8	7	7	0
Su	10	9	7	0
Y	6	2	5	0
Total $E + \frac{F}{2}$	72	47.5	30.5	4
%	6.0	6.8	5.8	0.7
Germless Seeds	62	7	4	2
%	5.2	1.0	0.8	0.3

[1] Weighted mean of varying periods of exposure.

The effect of the water-filtered radiation from the discharge tube upon the frequency of endosperm deficiencies and germless seeds, together with comparable results of filtered radiations of the mercury arc, are shown in table 1. *Wx* deficiencies are omitted from this table, since the seeds affected are in some cases included among the *C* deficiencies observed.

With each radiation the treatments applied included a rather wide range of dosage. The relation of dosage to genetic effectiveness cannot be determined accurately with the mercury arc because of uncontrolled fluctuations in intensity with temperature and other conditions. The discharge tube radiation is less variable. In treatments with the discharge

tube the doses ranged from $7^1/_2$ to 120 seconds, and the full dosage series was carried out in consecutive treatments during a two-hour period, with a complete repetition the following morning, using the same plant as pollen source and maintaining conditions as uniform as possible. The results are subject to a rather high probable error due to the small populations available, but they indicate that the relation of total frequency of deficiency to dosage is approximately linear. In table 1 the results at all doses used are summarized for each radiation, the weighted mean of exposure periods being given to permit comparison of the effect of the different radiations in equivalent exposures.

The relative frequency of loss of the various marker genes used does not differ materially in the trials with the discharge tube radiation and the various filtered radiations. In both the number of deficiencies is somewhat lower for *Y* than for the other genes tested, and the frequency of fractionals is distinctly higher for the aleurone color genes *A* and *C* than for the other markers. Unfortunately, the effects due to *A* and *C* losses cannot be separated in this mating. The results with *A* in the previous trials suggest that the preponderances of fractionals for aleurone color in this trial may be due chiefly to *A*. This is supported by the fact that only a small proportion of the fractional color losses reported in table 1 involve *Wx*, although trials with *c wx* stocks (with unfiltered ultra-violet radiation) had indicated that most *C* losses involve *Wx* also.

Although the relative frequency of entire and fractional losses apparently differs at different loci, the frequency of fractional deficiencies is clearly increased at all loci by the various ultra-violet treatments. This extends to additional loci the contrast previously noted between ultra-violet and x-ray treatment of the pollen in their effect on the frequency of fractional deficienes.

The genetic effect of the low doses tolerated with the discharge tube radiation was approximately equal to that of the relatively high doses of the filtered radiations. Considering the observed genetic effects in relation to the spectral distribution of energy in the radiations applied, it is evident that λ 2537 is much more effective than either λ 2967 or λ 3022 per unit of energy applied at the surface of the pollen grain.

The most striking difference in the trials of the relatively short wave-length radiation of the discharge tube and the longer wave-length filtered radiations is in their effect on the frequency of germless seeds. Comparing doses approximately equal in frequency of induced endosperm deficiencies, the percentage of germless seeds in excess of the control frequency was about nine times as large for the discharge tube radiation as for the filtered radiations. The difference is consistent in the various pollinations summarized in table 1, and is confirmed by additional comparisons in other matings. This indicates that the physical alterations in the treated pollen

which result in the production of germless seeds are essentially different from the alterations which result in induced deficiencies, and are relatively much less affected by the longer wave-lengths. The comparison of discharge tube and filtered radiations thus shows that much smaller doses of λ 2537 than of λ 2967 and λ 3022 are required for a given genetic effect, and that even with these smaller doses the shorter wave-length has a much more pronounced effect on the frequency of germless seeds.

The reduced tolerance to treatments with the shorter wave-lengths may be due in part to effects analogous to those resulting in germless seeds. The type of alteration in the sperm which results in failure of the embryo to develop, may, when it affects the other sperm, sometimes result in failure of development of the endosperm and thus of the seed as a whole. Such alterations would reduce the proportion of seed set, regardless of the presence of excess pollen, while changes affecting germination of the pollen grain, growth rate of the pollen tube, etc., would not affect the set if the amount of pollen not so affected were sufficient for fertilization of all of the ovules.

Comparison of the Effects of Ultra-Violet and X-ray Treatment.—The effects of ultra-violet treatment in these experiments have differed in various ways from the effects previously noted in similar experiments with x-ray treatment of pollen. The contrasts indicated were the following:

(1) Among the endosperm deficiencies induced, the proportion of fractionals is much higher with ultra-violet treatment than with x-rays.

(2) The proportion of germless seeds produced by ultra-violet treated pollen is much lower than that produced by x-rayed pollen. With the longest ultra-violet wave-lengths effective in inducing deficiency, the effect on frequency of germless seeds is almost inappreciable.

(3) There is no evidence of any increase in frequency of translocation under ultra-violet treatment.

(4) Among deficiencies affecting the F_1 plants, the proportion of haplo-viable deficiencies may be higher with ultra-violet than with x-ray treatment.

(5) Of the mutations induced by ultra-violet treatment, several occurred in germ cells in which apparently unrelated mutations or other germinal alterations also occurred.

All of these apparent contrasts are based on comparison of the results of experiments made at different times and with different stocks. Obviously they must be confirmed in strictly comparable trials before they may be considered conclusive. Such trials are now being made with various ultra-violet treatments in comparison with x-ray treatments covering a wide range of dosage. The range in dosage is necessary in order to distinguish between differences in effect due to spectral variations in activity and those which may be incidental to dosage differences. For example, if

translocations are rare following ultra-violet treatment and common following x-ray treatment, it does not follow necessarily that translocations are dependent upon some initial physical change induced by x-rays and not by ultra-violet. An alternative possibility is that translocations occur by deferred reattachment following chromosome breakage, as suggested by Stadler[1] and since the number of chromosome breaks is much smaller in the ultra-violet treated cells, the opportunities for interchange in reattachment are extremely rare. If so, x-ray doses producing the same number of chromosome breaks as the ultra-violet treatments used also should fail to increase appreciably the frequency of translocation.

Comparable data for x-ray and ultra-violet treatment are now available only for the effect on frequency of endosperm deficiencies and germless seeds. In connection with the trials of filtered radiations reported in the preceding paper, comparable pollinations were made with x-rayed pollen. The freshly dehisced anthers were treated in a shell vial just before pollination. The radiation was that emitted by a Coolidge tube operated at 140 K.V.P., with no filtration except that of the glass vial. The dose was 1333 r.

In comparing the effects of x-ray and ultra-violet treatment on the frequency of entire and fractional endosperm deficiencies, the deficient endosperms showing "recovery" are a source of some difficulty. "Recovery" is the term applied to the occurrence of small islands of non-deficient tissues in individuals otherwise deficient. Among the deficiencies induced by x-ray treatment, a small fraction, varying with the marker used, show recovery. For reasons previously discussed[1,2] these are considered a group distinct from the endosperm mosaics, and are ascribed to segmental deficiency affecting the endosperm as a whole followed by recovery of the segment in one or more cells in the course of endosperm development. In some instances, however, it is impossible to distinguish between recovery and mosaic individuals. If the development of the endosperm were a geometrically regular process, all mosaics due to loss occurring in the course of endosperm development would show the recessive character in sector of $^1/_2$, $^1/_4$, $^1/_8$, $^1/_{16}$, ... , depending on the cell generation in which the loss occurred, while recovery would be manifested by endosperms showing the recessive character in $^1/_2$, $^3/_4$, $^7/_8$, $^{15}/_{16}$, ... , depending on the cell generation in which the segment was restored to normal distribution and activity. But endosperm development is extremely irregular and consequently it is impossible to determine in individual cases the cell generation in which a loss or recovery occurred. In practice we draw an arbitrary line between the two groups, classifying the entire series $^{15}/_{16}$ to $^1/_{16}$ as fractionals and classifying definitely as deficiencies followed by recovery only those seeds in which the total fraction showing the dominant character is estimated as $^1/_{32}$ or less. Many of the seeds classified as frac-

tionals with large recessive sectors may be the result of the same phenomenon as those in the recovery class. In comparing the effects of x-ray and ultra-violet treatments, which appear to differ in their relative effect on entire and fractional deficiencies, it is therefore desirable to distinguish between fractionals of the various classes. In classifying the seeds, the size of the recessive sector was carefully estimated in each fractional ex-

TABLE 2

COMPARATIVE EFFECT OF X-RAYS AND ULTRA-VIOLET RADIATION ON ENDOSPERM DEFICIENCIES AND GERMLESS SEEDS

	X-RAYED POLLEN (1333 r)	ULTRA-VIOLET-TREATED POLLEN FILTER A	FILTER B	FIFTER C	CONTROL
No. Seeds Examined	1901	3007	3656	4449	1941
Mean Exposure Period (Min.)	4.55	2.78	4.39	7.48	.....
Deficiencies					
A. Entire					
(1) Without Recovery					
A	128	50	75	23	1
Pr	20	31	33	15	2
Total (%)	7.79	2.69	2.95	0.85	0.15
(2) With Recovery ($^{31}/_{32}$ or more of recessive tissue)					
A	38	5	6	3	0
Pr	0	0	0	0	0
Total (%)	2.00	0.17	0.16	0.07	0.00
B. Fractional					
(3) $^{15}/_{16}$–$^{7}/_{8}$ recessive tissue					
A	23	16	22	9	1
Pr	0	0	1	0	0
Total (%)	1.21	0.53	0.63	0.20	0.05
(4) $^{3}/_{4}$–$^{1}/_{4}$ recessive tissue					
A	14	116	125	74	4
Pr	1	21	26	16	1
Total (%)	0.79	4.56	4.13	2.02	0.26
(5) $^{1}/_{8}$–$^{1}/_{16}$ recessive tissue					
A	14	28	26	10	2
Pr	2	3	2	2	1
Total (%)	0.84	1.03	0.77	0.27	0.15
Germless Seeds	340	45	22	10	5
%	17.89	1.50	0.60	0.22	0.26

amined. In table 2 the data on endosperm deficiencies are summarized in five classes, based on the extent of the tissue showing the recessive character.

The contrast in effect of the two kinds of radiation on fractional and entire endosperm deficiencies is clearly shown by these data. Comparing x-ray treatment with ultra-violet radiation *A*, for example, the dose of 1333 r produces about three times as high a percentage of entire endosperm deficiencies as the mean ultra-violet dose used, but (even if all deficiencies with recovery be included among the fractionals) a distinctly smaller

percentage of fractionals. The fractionals following ultra-violet treatment are symmetrically distributed about the $^1/_2$ class, but those following x-ray treatment are most frequent in group 3 ($^{15}/_{16}$–$^7/_8$) with a still larger number in the recovery class (group 2), corresponding to $^{31}/_{32}$ or a higher fraction of the endosperm recessive. This suggests that most of the apparent fractionals induced by x-ray treatment are in fact due to recovery, and that this treatment has little effect on the phenomenon responsible for fractionals of the type induced by ultra-violet treatment.

It is clear also that the relative effect of the two types of radiation on deficiencies at the two loci marked is widely different. As previously stated, the *Pr* deficiencies noted represent the frequency of deficiency for only half of the population, since the seed parent was heterozygous *Pr pr*. For "entire" endosperm effects (groups 1 and 2) the percentage of *A* and *Pr* deficiencies respectively under x-ray treatment was 8.73 and 2.10; under ultra-violet treatment *A*, 1.83 and 2.06. Results with the other ultra-violet radiations were similar, the total frequency of entire deficiencies of *Pr* being about equal to that of entire deficiencies of *A* under ultra-violet treatment.

Finally, the frequency of germless seeds produced by x-ray treatment of pollen is very much higher than that produced by ultra-violet treatment. In this connection it should be recalled that ultra-violet radiations of shorter wave-length produce a much higher proportion of germless seeds than the filtered radiations here reported (see table 1), but the percentage of germless seeds produced by x-ray treatment is higher than that produced by any ultra-violet radiation used in these experiments.

Summary.—Comparable trials of mercury discharge tube radiation (largely λ 2537) and filtered mercury arc radiations (largely λ 2967 and λ 3022 plus various genetically ineffective wave-lengths) indicated:

(1) The relative frequency of induced deficiency at the various loci tested does not differ appreciably for the longer and shorter wave-lengths.

(2) The maximum dose tolerated is much lower for the shorter wave-length.

(3) The frequency of induced deficiency per unit of energy applied at the surface of the pollen grain is much higher for the shorter wave-length.

(4) The difference between the wave-lengths compared in effect on the frequency of germless seeds is even more extreme. Comparing doses which induced deficiency in approximately equal frequencies, the shorter wave-length radiation produced several times as many germless seeds as the longer wave-length radiation.

Comparable trials of x-rays (1333 r) and filtered mercury arc radiations (various doses) indicated:

(5) Almost all x-ray-induced deficiencies affect the endosperm as a

whole, while a large proportion of ultra-violet-induced deficiencies are fractionals affecting approximately half of the endosperm.

(6) The frequency of entire endosperm deficiencies of *A* is much higher than that of *Pr* following x-ray treatment, but *A* and *Pr* deficiencies are about equally frequent following ultra-violet treatment.

(7) The frequency of germless seeds produced by x-rayed pollen was much higher than that produced by ultra-violet-treated pollen.

Additional contrasts between ultra-violet and x-ray effects indicated by these experiments but not yet tested in strictly comparable trials are listed in the text.

[1] Stadler, *Proc. 6th Int. Cong. Genetics*, **1**, 274–294 (1932).
[2] Stadler, these PROCEEDINGS, **16**, 714–720 (1930).

A TERMINAL INVERSION IN DROSOPHILA ANANASSAE

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The existence of inverted segments in chromosomes of wild-type populations of *Drosophila melanogaster* was determined genetically by Sturtevant[1] from the effects which such inversions have in reducing crossing-over. Following the more recent application of the salivary gland method for cytological analysis of chromosome aberrations, naturally occurring inversions have been identified in several species of *Drosophila*. Thus, Tan[2] and Koller[3] found that race *A* and race *B* of *D. pseudoobscura* differ in four inverted sections. Sturtevant and Dobzhansky[4] discovered inversions in wild populations of either race of *D. pseudoobscura* inhabiting different geographical regions. Dubinin, Sokolov and Tiniakov[5] found a number of inversions widely distributed in different populations of *D. melanogaster* and *D. funebris*. Frolova[6] observed inversions in the chromosomes of *D. repleta* and *D. sulcata*. In the present study four inversions, one of which involves a terminal section of a chromosome, were found in *D. ananassae*. All four occur in autosomes.

The somatic chromosome complement of *D. ananassae* De Meijere (*D. caribbea* Sturtevant) has been determined by examination of the large cells of the ganglia of the larvae.[7] There are three pairs of *V*-shaped autosomes and a pair of *V*-shaped *X*-chromosomes in the female (Fig. 1). In the male the sex chromosomes are an *X* and a *J*-shaped *Y*. In prophase cells of female larvae, the nucleolus is associated with the pair of shortest autosomes, and separates a small chromomere-like satellite from the bulk