THE INDUCTION OF CHROMOSOME ABERRATIONS IN TRADESCANTIA BY ULTRAVIOLET RADIATION¹

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DIRECT cytological studies of ultraviolet-induced chromosomal aberrations, in contrast to the mutagenic effects of this agent, have been few in number and limited in extent. The meager literature on the subject has been summarized by SWANSON and STADLER (1955), and it deals primarily with aberrations in *Tradescantia* pollen-tube chromosomes. In most other organisms possessing chromosomes suitable for cytological examination, induction of nuclear damage by ultraviolet radiation is extremely difficult owing to the strong absorption and scattering in this region by overlying tissues or cellular constituents. Notable exceptions to this state of affairs are the studies of STRAUB (1941), SLIZYNSKI (1942), and the recent paper of FABERGÉ (1956). The pioneer work on *Tradescantia* was done by SWANSON (1940, 1942, 1943, 1944), who observed simple chromatid deletions but no isochromatid or exchange aberrations after irradiation of growing pollen-tube cultures. Most of these investigations were carried out with monochromatic radiation of wave-length 2537 Å; in a few instances, mixed radiation in the near ultraviolet was used. After these studies by SWANSON, the subject has been largely ignored experimentally until the work by LOVEFACE (1954), a surprising neglect in view of the cytological suitability of *Tradescantia* chromosomes, the accessibility of the pollen or pollen tube nuclei to ultraviolet radiation, and the development of reliable and routine pollen culture techniques (BISHOP 1949; CONGER 1953). LOVEFACE (1954) treated *Tradescantia* dry pollen with 2650 Å ultraviolet radiation and succeeded in producing simple chromatid breaks, isochromatid breaks, and chromatid/chromatid interchanges, i.e., the customary types of chromatid breaks induced by ionizing radiation, as well as complete fragmentation of entire chromatid arms and, often, entire chromosomes. The contrast between the findings of SWANSON on the one hand and those of LOVEFACE and the present authors on the other are not too surprising in view of the fact that the ultraviolet treatments were given under quite different conditions, i.e., growing pollen tubes versus dry pollen.

The investigations cited were primarily qualitative and were concerned mainly with the question of whether ultraviolet radiation is able to induce chromosome breakage. After LOVEFACE's observation (1954) that ultraviolet treatment of dry pollen does result in chromosomal aberrations in the pollen tube division in *Tradescantia*, a further investigation of this phenomenon, including the study of factors governing ultraviolet-induced chromosomal damage and a comparison of the results of ultraviolet treatment with those produced by ionizing radiation, has appeared highly desirable. Brief preliminary accounts of some aspects of these studies have been reported in abstract form (KIRBY-SMITH 1954; KIRBY-SMITH and CRAIG 1955).

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The present paper represents the complete account of our work in these problems. It also includes a semiquantitative determination of the action spectrum for chromosomal breakage by ultraviolet radiation. Among the requirements for a reliable wave-length experiment of this type, as well as for other more quantitative studies, are: (1) a knowledge of the time-intensity or reciprocity relations; (2) definite proof that breakage by ozone is not a contributing factor to the results; and (3) a determination of the role of any possible photorecovery processes. A study of these possible modifying factors has been made in addition to work on the larger problem of the effects of wave length and dose.

EXPERIMENTAL METHODS

Dry pollen from *Tradescantia paludosa* Anderson and Woodson, Sax clone 3, was used in all experiments. The handling of the pollen was facilitated and clumping was prevented by desiccating the pollen over anhydrous CaSO_4 for approximately thirty minutes immediately after collection. After drying, the pollen was dusted in a monolayer over the surface of a glass slide 0.5 x 2.0 cm. At this point, the preparations were examined under a low-power dissecting microscope and any clumps of pollen were removed with a needle, thus ensuring that no pollen grains were shielded from the incident radiation. The slides were then placed in the irradiation chamber shown schematically in figure 1, and the pollen was subjected to ultraviolet treatment.

All irradiations were carried out at constant temperature and humidity. The relative humidity was controlled at approximately 50% by bubbling a stream of air through a saturated solution of $\text{Ca}(\text{NO}_3)_2$, thoroughly filtering to remove any entrained droplets, and gently blowing the air stream over the pollen as indicated in figure 1. Room temperature was controlled within the range 25 to 27°C. The thick aluminum block on which the material was placed for irradiation served to minimize ambient temperature fluctuations by virtue of its large thermal inertia. All treatments were carried out in darkness so that possible photoreactivation processes would be minimized. The rather wide day-to-day variation in the biological response

FIGURE 1.—Ultraviolet irradiation of dry pollen. E—Monochromator Exit Slit; F—Front Surface Mirror; B—Aluminum Block; S—Glass Slide; P—Pollen Monolayer; W—Fluorite Window.

of pollen to radiation previously encountered in X-ray experiments was largely avoided by these procedures and, in most cases, it has been possible to lump the results of a number of similar experiments performed at different times.

Irradiations were carried out using a monochromator with crystal quartz optics and relative aperture of f4. This instrument is relatively free from stray scattered radiation and permits slits up to 1.0 mm to be used at most wavelengths without appreciable scattered energy at the exit position. In all the present work a slit width of 0.8 mm or less was employed.

The sources used were AH4 lamps with the outer glass bulbs removed or the CH3 lamps with ultraviolet-transmitting outer envelopes. The AH4 is a better source in the 2650 Å region, although the CH3 lamp may be preferred as a radiation source in the near ultraviolet down to a wave length of 2804 Å.

The intensity of radiation delivered to the surface of the pollen grains was measured with a thermopile and galvanometer whose response was routinely calibrated against a standard lamp. The material to be irradiated was placed a few centimeters from the exit slit of the monochromator at a point where the radiation field was slightly in excess of 0.5 x 2.0 cm. Exploration of this field with the thermopile showed it to be uniform to less than 5% over the area used. The choice of the field dimensions quoted was dictated by considerations of minimum pollen sample size necessary to obtain significant numbers of scorable metaphase configurations in the processed cultures. With this area, the intensity obtained gave adequate biological effects after a short exposure at the most effective wave lengths; e.g., 20 minutes' exposure for a dose of 1×10^6 ergs/cm² at 2650 Å.

Studies of photoreactivation after ultraviolet irradiation were carried out by two different experimental arrangements. In the first of these, an AH4 mercury arc lamp was used in its original glass envelope mounted in a pyrex water jacket to remove undesirable infrared and ultraviolet energy. Any residual ultraviolet radiations transmitted by this system, including the 3650 Å lines of mercury, were completely removed by a Corning filter # 3389. With this arrangement, photoreactivation doses of 2×10^6 ergs/cm² to 5×10^8 ergs/cm² were given the dry pollen subsequent to ultraviolet treatment. In an attempt to discover if small amounts of 3650 Å ultraviolet radiation alone (at lower dose levels than could cause a measurable aberration rate) could result in any photorecovery, a second set of photoreactivation experiments were carried out with the monochromator assembly shown in figure 1.

Immediately after irradiation, the dry pollen was sowed on a lactose-agar medium containing colchicine and cultured 18 to 20 hours, after methods described by BISHOP (1949) and CONGER (1953). Feulgen-stained slides were made of these cultures and the chromatid aberrations scored in metaphase of the pollen tube division. All scoring was done by one person (DLC).

RESULTS AND INTERPRETATIONS

The complete experimental results are summarized in tables 1 through 5. These tables contain the dosage-aberration frequency data for (1) some typical dose-aberration curves, (2) the time-intensity, or reciprocity law studies, (3) the air-nitrogen comparison experiments, (4) the attempts to observe photoreactivation

processes, and (5) the relative effects of ultraviolet radiation of different wave lengths. Although an attempt has been made to keep the tables completely self-explanatory, some additional amplification or emphasis is needed on a few points.

Without exception, all doses were determined experimentally in a direct manner and are expressed in terms of ergs per square centimeter of ultraviolet radiation incident on the surface of the pollen grains. In all cases (with the exception of some of the photoreactivation experiments in which the filter technique was used), the dosage figures refer to monochromatic radiation of the wave length indicated. For the special photoreactivation studies, where a filter was used, the incident energy fell within the wave-length region 4000 to 6000 Å with peaks due to mercury arc lines at 4047, 4385, and 5460 Å.

In the light of the very large cytoplasmic absorption of photochemically active radiation, it is not surprising that several general physiological effects are evident in the cultured pollen, in addition to the chromosomal effects. One property of most of the ultraviolet-treated material was a much poorer germination and growth than after an amount of ionizing radiation resulting in equivalent chromosomal breakage. The results also indicate that ultraviolet-treated chromosomes are much longer and tend to clump together more than those subjected to ionizing radiation.

Chromosome fragmentation has severely limited the range over which dose-aberration frequency curves can be analyzed. This can be seen in the data of table 1 which shows the saturation in the aberration frequencies encountered in the higher doses. At still higher doses, fragmentation becomes the dominant form of chromosome damage. Under these conditions, so many cells are unscorable in the usual sense that the dose-aberration frequency curves tend to reach a limiting plateau, and scorable aberrations may even decrease with additional dose. Fragments are so numerous that any quantitative observation of them is impossible. For these reasons all experimental points used for comparison were chosen in the lower dosage ranges, where fragmentation is not a serious factor. The production of chromosome fragments by ultraviolet treatment is in sharp contrast to the effects of ionizing irradiation where, except for α -particle irradiation, very few cases of fragmentation are observed.

The data for wave-length dependence summarized in table 5 were plotted in figure 2. The curves shown here give the rough shape of the action spectrum in terms of the energy incident on the pollen grains.

TABLE 1
Variation of aberration frequency with dose

Expt. no.	UV dose [(ergs/cm ²) × 10 ⁶]	No. of cells scored	Percentage normal	No. of aberrations per cell		
				Cd.	Iso.	Fragmented chromosomes
17, 2650 Å	0.52	400	70	0.24	0.16	0.03
	1.01	200	58	0.42	0.22	0.12
	1.52	200	49	0.50	0.29	0.18
8, 2804 Å	0.97	150	68	0.28	0.15	0
	1.99	100	47	0.57	0.44	0.19
	3.44	92	28	0.56	0.65	0.53

TABLE 2
Time-intensity data for ultraviolet-induced aberrations

Expt. no.	Intensity (ergs/cm ² /sec)	UV dose, at 2650 Å [(ergs/ cm ²) × 10 ⁶]	No. of cells scored	Percentage normal cells	No. of aberrations per cell		
					Cd.	Iso.	Ex.
4	925	1.18	135	52	0.36	0.41	0.01
	93.1	1.12	25	44	0.32	0.48	0
7	958	1.15	100	41	0.61	0.49	0.01
	95.3	1.14	100	32	0.49	0.44	0
17	1092	0.51	200	71	0.22	0.17	0.01
	118	0.53	200	69	0.26	0.14	0.01

TABLE 3
Aberration frequencies for pollen irradiated in air and in nitrogen

Expt. no.	Treatment	UV dose, at 2650 Å [(ergs/ cm ²) × 10 ⁶]	Cells scored	Percentage normal cells	No. of aberrations per cell			Percentage inc. iso.
					Cd.	Iso.	Ex.	
4	Air	1.18	135	52	0.36	0.41	0.01	44
	N ₂	1.15	150	53	0.37	0.39	0	66
17	Air	1.01	200	58	0.42	0.22	0.01	46
	N ₂	0.98	200	56	0.37	0.24	0	65
34	Air	0.98	250	44	0.37	0.22	0.02	48
	N ₂	1.00	300	35	0.45	0.21	0.01	62

TABLE 4
Results of photorecovery studies

Expt. no.	UV dose, at 2650 Å (ergs/cm ²)	Photo- reactivation dose, (ergs/cm ²)	Photo- reactivation wave-length Range (Å)	No. of cells scored	Percentage normal	Aberration frequency per cell		
						Cd.	Iso.	Ex.
25	0.96 × 10 ⁶	None		150	25	0.39	0.49	0.05
	0.98 × 10 ⁶	5.37 × 10 ⁸	4000	150	25	0.35	0.55	0.06
	None	5.37 × 10 ⁸	4000	150	95	0.02	0.03	0
31	1.0 × 10 ⁶	None		250	31	0.52	0.38	0.06
	1.0 × 10 ⁶	2.98 × 10 ⁶	4000	200	34	0.56	0.30	0.02
	1.0 × 10 ⁶	1.49 × 10 ⁷	4000	200	25	0.58	0.40	0.06
	0.99 × 10 ⁶	1.47 × 10 ⁶	3654	200	28	0.52	0.38	0.09
32	None	1.01 × 10 ⁷	3654	225	95	0.04	0.01	0
	None	1.99 × 10 ⁶	3654	50	94	0.06	0	0
	None	3.99 × 10 ⁶	3654	100	96	0.05	0	0
	1.0 × 10 ⁶	None		250	26	0.44	0.33	0.07
	1.0 × 10 ⁶	0.82 × 10 ⁷	3654	200	23	0.48	0.40	0.10

Before any real understanding of the role of ultraviolet radiation in the induction of chromosome breakage can be attained, a study of the parameters governing such damage must be made. It is particularly important that the factors influencing of modifying chromosomal breakage in pollen be determined and evaluated in much the same manner as has been done already for ionizing radiation. Taken alone, this

TABLE 5
Variation of aberration frequency with wave length

Wave length Å	UV dose (ergs/cm ²)	No. of cells scored	Percentage normal	No. of aberrations per cell				RBE for isoch. aberrations normalized to unity at 2545
				Cd.	Iso.	Ex.	Fragmented chromo- somes	
3654	3.01×10^7	250	88	0.10	0.03	0	0	0.024
	1.01×10^7	225	95	0.04	0.01	0	0	0.024
3130	3.25×10^7	114	92	0.02	0.06	0	0	0.043
3020	7.09×10^6	186	57	0.35	0.10	0.02	0.005	0.06
	3.57×10^6	300	69	0.27	0.09	0.03	0	0.06
2804	1×10^6	272	64	0.29	0.17	0	0.04	0.40
2650*	1×10^6	2036	40	0.43	0.33	0.04	0.61	0.79
2545	0.5×10^6	100	60	0.28	0.21	0.02	0.12	1.00
2483	0.98×10^6	350	57	0.39	0.20	0.07	0.25	0.58
2350	1.0×10^6	300	76	0.17	0.11	0.05	0.007	0.26

* Pooled data.

FIGURE 2.—Action spectrum for ultraviolet-induced chromosomal aberrations and nucleic acid absorption spectrum. Curve A—Action spectrum for ultraviolet-induced isochromatid aberrations. Curve B—Absorption spectrum for a nucleic acid (HOLLAENDER, GREENSTEIN and JENNETTE 1941). Ordinate units arbitrarily adjusted for equal maximum effect.

approach should yield much information for aberrations induced by ultraviolet radiation; the comparison of the results of this agent with those produced by ionizing radiation should lead to a better understanding of both phenomena. Before proceeding with the discussion of the specific individual experiments dealing with time-intensity effects, photoreactivation, air-nitrogen breakage ratios and action spectra studies, it is of interest to consider some general aspects of the data as a whole.

The experimental results show very clearly that ultraviolet radiation leads to chromosome breakage of the same types as those found for ionizing radiation. It must also be obvious that these ultraviolet-induced aberrations are the results of activation processes, since the energies available (i.e., the primary energy of the photons) are close to 5 volts for 2500 Å radiation. This is much less than the approximately 15 ev required for ionization. These figures might appear to show that activa-

tions of about 5 ev play an important part in the production of aberration by ionizing particles, for, of the 34 ev expended in producing an ion pair, almost 20 ev are dissipated in the form of activation energy. A closer examination of the ultraviolet induction of aberrations reveals that this is probably not the case, at least for radiation of wave lengths greater than 2200 Å.

This can be qualitatively shown by comparison, on an energy basis, of the effects of ultraviolet and X-rays. Experimentally, a surface dose of 2650 Å ultraviolet radiation of 10^6 ergs/cm² produces approximately the same number of chromatid or isochromatid aberrations as 100r of cobalt gamma rays. UBER's data (1939) for the absorption of ultraviolet radiation in maize pollen walls and cytoplasm was used for computation of a rough value for the depth dose relations in *Tradescantia* pollen.² For 2650 Å radiation, and if the generative nucleus is assumed to be located along the central axis of the pollen grain, a figure of $\sim 10^7$ to 10^8 ergs/g is obtained for a surface exposure of 10^6 ergs incident on the pollen grain.

Since the equivalent biological dose of gamma rays or hard X-rays is 100r or $\sim 10^4$ ergs/g, for equal energy absorbed in the sensitive material, gamma rays are approximately 10^3 to 10^4 times as effective as ultraviolet in the production of chromosome breakage. Similar results for the depolymerization and reduction in viscosity of deoxyribonucleic acid solutions have been observed by FISHER (Personal communication 1956) in this Laboratory. Thus the relatively small amount of energy available for activation in material treated with ionizing radiation can be expected to produce a negligible biological effect compared with ionization. These arguments hold only for activations of less than 5 or 6 ev; it may be that higher energy activations have a higher relative biological efficiency. If a suitable material can be found,

² This estimate is arrived at by using UBER's (1939) absorption coefficients for maize pollen, assuming the absorption of ultraviolet radiation in *Tradescantia* pollen to be roughly equivalent to that in maize. The generative nucleus of the *Tradescantia* pollen grain is considered, on the average, to be at the center of the pollen grain, i.e., approximately 8 μ below the surface; the nucleus is also taken to be 4 to 5 μ in diameter. UBER's measurements on maize indicate a 20% transmission for the pollen wall and a 20% transmission for an 8- μ -thick layer of cytoplasm at 2650 Å, the wave length of maximum absorption. If these data are applied to *Tradescantia*, it is seen that $(0.20) \times (0.20)$ or 4% of the incident radiation reaches the generative nucleus.

The fraction of this radiation absorbed in the nucleus may be readily calculated from the usual exponential relation:

$$I/I_0 = e^{-\mu x}$$

where, I_0 = radiation incident on the pollen nucleus, I is the radiation transmitted through the pollen nucleus, and μ is the linear absorption coefficient in microns⁻¹ calculated from UBER's results, $0.2 \mu^{-1}$.

By these values for the *Tradescantia* generative nucleus (taken to be 4 microns in thickness),

$$I/I_0 = e^{-0.2 \times 4} = 0.45.$$

Thus, of the 10^6 ergs/cm² originally incident in the pollen grain,

$$10^6 \text{ ergs/cm}^2 \times 0.04 \times 0.45 = 1.8 \times 10^4 \text{ ergs/cm}^2$$

is absorbed in the 4- μ -thick generative nucleus. In terms of ergs per gram and if the density of the nucleus is assumed to be unity, this amount of energy becomes

$$\frac{1.8 \times 10^4 \text{ ergs/cm}^2}{4 \times 10^{-4} \text{ g/cm}^2} = 4.5 \times 10^7 \text{ ergs/g.}$$

and some acceptable means for delivering ultraviolet in the vacuum range (1500 Å), a complete answer to this question may be achieved.

In contrast to the similarity in aberration types induced by ionizing and ultraviolet radiations, the relative numbers of the different aberrations produced by the two kinds of radiation are quite different. In pollen treated with 250-kvp X-rays, and at low doses (~ 100 r) where the exchange dose-effect relations have not departed appreciably from a linear relation the ratio of chromatid to isochromatid breaks to chromatid/chromatid interchanges is approximately 1:3:1 (KIRBY-SMITH and DANIELS 1953); for this material treated with ultraviolet of wave length 2650 Å, the aberration frequencies are in the approximate ratios of 1:0.5:0.05, respectively (see tables). An additional difference is found in the numbers of the various types of isochromatid aberrations induced by ultraviolet as contrasted with X-rays. For ultraviolet treatment, the proportion of incomplete isochromatid aberrations found, i.e., isochromatid aberrations that do not show the usual fusion at both ends, is far greater than that observed for pollen irradiated with X-rays. CONGER (1955) has found that about 18% of isochromatid aberrations are incompletely fused in microspores treated with X-rays in air and nitrogen. The figures in table 3 show this to be approximately 45% in air and 65% in nitrogen for pollen exposed to ultraviolet radiation of wave length 2650 Å. These findings, the low frequency of interchanges relative to chromatid and isochromatid deletions and the high percentage of isochromatid aberrations that are incomplete, indicate that the ends of chromosomes broken by ultraviolet do not rejoin to the same extent as those produced by ionizing radiation. The extremely low aberration frequencies found for ultraviolet-induced chromatid/chromatid exchanges also explain very clearly and dramatically the infrequent occurrence of translocations so often reported in genetic studies of the action of ultraviolet radiation.

In addition, if fusion is less in breaks produced by ultraviolet than in breaks resulting from ionizing radiation, more aberrations per primary break can be expected from ultraviolet than from ionizing radiation. Since the results do show definitely that fusion is less for ultraviolet-induced breaks, the number of primary breaks per erg is even less than previously calculated (see footnote).

Considering the greatly different primary physical action of these two separate agents, ionization and excitation, major differences in the nature of the broken ends is not surprising. Excitation events, such as those induced by ultraviolet absorption, leading to chromosome breakage indicates that similar processes accompanying or resulting indirectly from ionizing radiation may be an important, or potentially important, pathway to breakage after treatment with these much more energetic agents. Conversely, the relatively small percentage of experimentally observed isochromatid aberrations due to ionizing radiation that are incomplete may be construed as evidence that the excitation process is at least not dominant in this case.

Time-intensity studies

The data accumulated in these experiments designed to determine the effects of varying ultraviolet intensity at constant dose are shown in table 2. These investigations show that the reciprocity law holds over the approximately 10:1 range in

intensities that could be studied conveniently by our existing experimental arrangements. The establishment of the validity of the reciprocity law allows a comparison of the effects of ultraviolet radiation of different wave lengths to be made independent of intensity, and greatly simplifies the practical problems encountered in constructing an action spectrum. It has been possible to make measurements of the time-intensity relations over such a range, using the mercury emission lines at 2537 and 2650 Å, both of which are close to the maximum biologically effective wave length for chromosome breakage. Although other lines in the intermediate pressure mercury spectrum are many times as intense as these, i.e., 3130 or 3650 Å, the relative biological effectiveness of energy of these wave lengths is so small that, even at the higher intensities, the time required to deliver adequate doses for a reasonable aberration frequency has prevented reciprocity law checks at other wave lengths.

Considering the relatively low exchange frequency and the small number of re-fused chromatid breaks as exemplified in the isochromatid data, one would not expect to find a great dependence on intensity for ultraviolet-induced aberrations. In other words, one-hit aberrations such as chromatid aberrations and the great majority of isochromatid aberrations are, or should be, independent of intensity, and exchanges (the intensity-dependent aberrations) are too infrequent for a practical study. If the dose is increased to a figure that should result in an appreciable interchange frequency, complete fragmentation of chromosomes or parts of chromosomes becomes so prevalent that no quantitative scoring of the results is possible.

Air-nitrogen studies

The results given (table 3) have shown that there are no significant variations in the over-all frequencies for the general types of aberrations (chromatid and isochromatid) that can be studied quantitatively, for pollen treated in air and in nitrogen. In addition these data show that within the isochromatid class the proportion of isochromatid breaks that are incomplete is much greater for pollen irradiated in nitrogen than for that treated in air. This increase, from approximately 45 to 65% incomplete, is in good agreement with the hypothesis that breaks produced in nitrogen are less likely to rejoin than those produced in air. The increase is also in agreement with results for chromatid deletions in microspores treated with X-rays in air and in nitrogen (CONGER, personal communication, 1956). The additional experimental observation that equal numbers of chromatid aberrations are produced in pollen irradiated in air and in nitrogen is in complete disagreement with such a simple restitution theory. This apparent paradox can be resolved by an additional assumption that fewer breaks are initially produced in nitrogen than in air. The present experimental results thus support a tentative hypothesis in which (1) fewer breaks are produced in nitrogen than in air, and (2) these breaks induced in nitrogen are less likely to rejoin than the ones produced in material irradiated in air. This argument contains implicitly the underlying assumption that at least some of the photochemical processes involved in oxygen and in nitrogen are quite different.

Since no more aberrations are produced in pollen irradiated in air than in pollen treated in nitrogen, it appears that ozone has not been responsible for ultraviolet-induced breakage in the present experiments. These data furnish additional evidence

that ozone or ozone-producing wave lengths have been prevented from reaching the biological material.

Photorecovery processes

No photorecovery effects have been demonstrated in experiments yet performed. Table 4 shows typical results covering a wide range of dose in the wave-length region in which photorecovery has been reported in other materials. The high doses used in these experiments did not include radiation of wave length 3650 Å or less, since a very small amount of chromosomal breakage appears to have been produced at these wave lengths. Although considerable effort was made to eliminate stray scattered radiation of wave length shorter than 3650 Å, a slight possibility remains that the breakage attributed to 3650 Å may be the results of spectral impurities. Two experiments with very low doses in the region 10^6 to 10^7 ergs/cm² (doses that are too small to show the apparent breakage by 3650 Å radiation) also failed to show photorecovery.

Action spectrum for chromosome breakage

Having demonstrated within limits the validity of the reciprocity relations, proved that ozone is not a contributing factor in any of the present work, and showed the photorecovery processes (if present at all) are minimal effects, we have been able to proceed with less uncertainty to an investigation of the action spectrum for chromosome breakage (table 5). Owing to the possible although slight variation from day to day in pollen sensitivity, irradiations have been carried out with at least two and usually three different ultraviolet wave lengths. In this manner, by always having relative measurements for several wave lengths, it has been possible to correct for the small remaining day-to-day variations in sensitivity and construct a complete curve showing the dependence of breakage on wave length. Since only a small amount of pollen, usually enough for only one or two slides, could be treated in any one irradiation, many experiments were performed repeatedly to obtain enough metaphase figures for statistical significance.

Table 5 shows the results of a number of these independent experiments. In all but a very few experiments, irradiation of a pollen sample with a dose of 1×10^6 ergs/cm² or radiation of wave length 2650 Å served as a standard control point. No significant differences were noted and the 2650 Å results have been lumped as shown in the table. A curve (A) showing chromosome breakage as a function of wave length derived from these data is shown in figure 2. The absorption spectrum (curve B) for a nucleic acid (HOLLAENDER, GREENSTEIN, and JENRETTE, 1941) is included for comparison.

In all the work presented here, the ultraviolet dose considered is that dose striking the surface of the pollen grains, not the actual dose delivered to the nucleus or particular chromosomes. In the absence of definite absorption coefficient for *Tradescantia* pollen walls and cytoplasm, the action spectrum shown has been plotted in terms of this surface exposure. An examination of the results of UBER (1939) for ultraviolet absorption in maize pollen indicates that no major change in the general shape of the action spectrum is to be expected on application of these corrections. From

these considerations, the curve shown in figure 2 may be assumed to represent, to a good first approximation, the true action spectrum for ultraviolet-induced chromosomal breakage.

It is interesting to note that the curve (A, Fig. 2) agrees quite well with the results of STADLER and UBER (1942) for endosperm mosaics produced by ultraviolet irradiation of maize pollen. A slight discrepancy does exist in the wavelengths of maximum effectiveness between the present results for chromosome breakage and those reported by KNAPP *et al.* (1939) for genetic alteration in *Sphaerocarpus donnellii*. More precise data for the absorption and scattering of ultraviolet radiation in *Tradescantia* pollen and in *Sphaerocarpus* as well will be required before this difference can be considered significant.

SUMMARY

Dry pollen of *Tradescantia paludosa* was irradiated with monochromatic ultraviolet light of various wave lengths, and the aberrations present at the pollen tube division were studied.

Investigation of the time-intensity relations has shown that for equal doses, the intensity of incident radiation may be varied by at least a factor of 10 with no change in the frequencies for one-hit aberrations. Two-hit aberrations are too infrequent for a practical study.

Irradiation of pollen in air and in nitrogen has indicated no significant differences in the number of chromatid and isochromatid aberrations for the two treatments. This lack of an oxygen effect for ultraviolet-induced breakage is unlike the situation for ionizing radiations. The observation that the same aberration frequencies are obtained in nitrogen as in air indicates that ozone has not been responsible for the breakage in these experiments. Although the over-all frequencies were the same, the percentage of incompletely fused isochromatid aberrations increased from approximately 45 for material irradiated in air to 65 for that treated in nitrogen.

A wide range of doses of wave lengths 4000 to 6000 Å and doses of 3650 Å (smaller than that required to produce breakage) have given no photorecovery of ultraviolet-induced chromosomal damage in the dry pollen.

An action spectrum for chromosome breakage in dry *Tradescantia* pollen by ultraviolet light covering the range 3650 to 2350 Å has been obtained and is in good agreement with nucleic acid absorption curves.

LITERATURE CITED

- BISHOP, C. J., 1949 Pollen tube culture on a lactose medium. *Stain Technol.* **24**: 9-12.
CONGER, A. D., 1953 Culture of pollen for chromosomal analysis at the pollen tube division. *Stain Technol.* **28**: 289-293.
CONGER, A. D., 1955 Discussion in Symposium on genetic recombination. *J. Cellular Comp. Physiol. Suppl.* **2** **45**: 309-312.
FABERGÉ, A., 1956 The analysis of induced chromosome aberrations by maize endosperm phenotypes. *Z. Ind. Abst. Vererb.* **87**: 392-420.
HOLLAENDER, A., J. P. GREENSTEIN, and W. V. JENRETTE, 1941 The effects of ultraviolet radiation on sodium thymonucleate. *J. Natl. Cancer Inst.* **2**: 23-28.
KIRBY-SMITH, J. S., 1954 Some aspects of chromosome breakage in *Tradescantia* by ultraviolet radiation. *Proc. First Intern. Photobiol. Congr. (4th Intern. Light Congr.)*, pp. 138-139. H. Veenman & Zonen, Wageningen.

- KIRBY-SMITH, J. S., and D. L. CRAIG, 1955 Some aspects of ultraviolet-induced chromosome aberrations in *Tradescantia* pollen. *Genetics* **40**: 579.
- KIRBY-SMITH, J. S., and D. S. DANIELS, 1953 The relative effect of X-rays, gamma rays, and beta rays on chromosomal breakage in *Tradescantia*. *Genetics* **38**: 375-388.
- KNAPP, E., A. REUSS, O. RISSE, and H. SCHREIBER, 1939 Quantitative Analyse der Mutationsauslösenden Wirkung Monochromatischen U.V.-Lichtes. *Naturwissenschaften* **27**: 304.
- LOVELACE, ROBERTA, 1954 Chromosome shattering by ultraviolet radiation (2650 Å). *Proc. Natl. Acad. Sci. U. S.* **40**: 1129-1135.
- SLIZYNSKI, B. M., 1942 Deficiency effects of ultraviolet light in *Drosophila melanogaster*. *Proc. Roy. Soc. Edinburgh B* **61**: 297-315.
- STADLER, L. J., and F. M. UBER, 1942 Genetic effects of ultraviolet radiation in maize. IV. Comparison of monochromatic radiations. *Genetics* **27**: 84-118.
- STRAUB, J., 1941 Chromosomenmutationen nach U.V. Bestrahlung. *Naturwissenschaften* **29**: 13-15.
- SWANSON, C. P., 1940 A comparison of chromosomal aberrations induced by X-ray and ultraviolet radiation. *Proc. Natl. Acad. Sci. U. S.* **26**: 366-373.
- 1942 Effects of ultraviolet treatment on the pollen tube chromosomes of *Tradescantia*. *Genetics* **27**: 491-503.
- 1943 Differential sensitivity of prophase pollen tube chromosomes to X-rays and ultraviolet radiation. *J. Gen. Physiol.* **26**: 485-494.
- 1944 X-ray and ultraviolet studies on pollen tube chromosomes. I. The effect of ultraviolet (2537 Å) on X-ray induced chromosomal aberrations. *Genetics* **29**: 61-68.
- SWANSON, C. P., and L. J. STADLER, 1955 The effect of ultraviolet radiation on the genes and chromosomes of higher organisms. *Radiation Biology* Edited by A. Hollaender. Vol. II, pp. 249-284. McGraw-Hill Book Co., Inc. New York.
- UBER, F. M., 1939 Ultra-violet spectrophotometry of *Zea mays* pollen with the quartz microscope. *Am. J. Botany* **26**: 799-807.

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