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*THE EFFECT OF OXYGEN ON THE FREQUENCY OF X-RAY INDUCED CHROMOSOMAL REARRANGEMENTS IN TRADESCANTIA MICROSPORES**

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The recent studies of Thoday and Read,[1] using root tips of *Vicia faba*, indicate that the availability of oxygen to cells is a very important factor in radiosensitivity. Under anaerobic conditions, sensitivity to x-rays is markedly reduced, as measured both by inhibition of growth and by frequency of anaphase figures showing chromosome aberrations. The results of Hayden and Smith[2] on x-radiation of barley seeds in a vacuum also suggest that the absence of oxygen results in reduced radiosensitivity. In view of these results it seemed of interest to test the effect of oxygen on the frequency of x-ray induced chromosomal rearrangements in Tradescantia, especially since there is already available a wealth of information on radiation effects in this organism as a result of the pioneer investigations of Sax[3] which, together with those of other investigators, have been summarized by Lea[4] and by Catcheside.[5]

Materials and Methods.—Tradescantia paludosa Anderson and Woodson, clone 5 of Sax, was used in all experiments. Observations were made at the first postmeiotic mitosis in the microspore by means of acetocarmine smear preparations at the four- to five-day interval following treatment, at which time only chromosome-break types are present. The principal aberration types analyzed at this period are interchanges (dicentrics and centric rings). In addition, interstitial deletions (intercalary deletions or isodiametric fragments) are numerous. Acentric rings and terminal deletions were also recorded but, because of their comparative rarity, were not used in later calculations.

The x-ray source, a Coolidge self-rectifying tube with tungsten target was operated at 250 kvp. and 15 ma. The inherent filtration was equivalent to 3 mm. aluminum. In addition, all exposures were made inside a lucite exposure chamber whose top was $^1/_4$-inch thick. The inflorescences were inserted in an upright position through holes in a very thin lucite platform in the center of the box in order to insure uniform exposures and to avoid back-scattering. Dosages were measured inside the exposure chamber at the position of the inflorescences with a 100-r Victoreen thimble ionization chamber recently calibrated by the U. S. Bureau of Standards. In all experiments, unless otherwise noted, the dosage rate was constant, 45 r/min.–different total dosages being administered by increasing the time of exposure.

In order to make exposures in different gases, inflorescences were first placed in a suction flask, evacuated with a water pump for approximately two and one-half minutes, and the appropriate gas then admitted into the flask. This procedure was repeated five times. The purpose of this operation was to remove the air enclosed by the sepals and petals of the buds. The inflorescences were then quickly placed in the lucite exposure chamber, which could be made airtight, the air evacuated and the appropriate gas admitted. This operation was performed twice. The lucite chamber was next placed in the x-ray machine and the inflorescences irradiated at room temperature. After exposure, the inflorescences were kept in the gas chamber for approximately ten minutes, following which they were removed, reëvacuated in the suction flask and air permitted to diffuse in. This procedure was repeated five times, as previously. Commercial cylinders of the various gases were utilized, the indicated purity in all cases being at least 99.5%.

In the first control experiment in which inflorescences were exposed to x-rays in air inside the lucite chamber, the same initial series of evacuations was carried out, except that air rather than some other gas was permitted to enter the flask following each evacuation. A repetition of this control experiment in air without prior evacuation of the buds gave essentially similar results indicating that the evacuation procedure itself did not modify the results. Observations of other control buds, exposed to atmospheres of nitrogen, oxygen and other gases in an identical manner but not x-rayed, indicated that exposure to the gas alone did not result in any detectable cytological abnormalities.

In general from 10 to 15 inflorescences were exposed at each treatment and as many slides as possible made at the four- and five-day interval following irradiation. The aberration frequency for each dosage was calculated from the total number of interchanges and interstitial deletions recorded in either 50 or 100 cells on 3 to 15 (average ca. 7) slides. Chi-square tests did not reveal any excess variation among slides from one treatment

and standard errors were calculated by the method discussed by Catcheside, Lea and Thoday.[6]

Results and Discussion.—The results of comparative experiments in which inflorescences were irradiated in air, in nitrogen and in oxygen are presented in table 1 and shown graphically in figures 1 and 2. All the data in experiment 1 (exposures in air) were obtained by one investigator (N. H. G.). Combined data from the scorings of both investigators for interchanges (dicentrics and centric rings) are given in all succeeding experiments since the scorings of these aberration types by both investigators are quite comparable. However, there is considerable individual variation

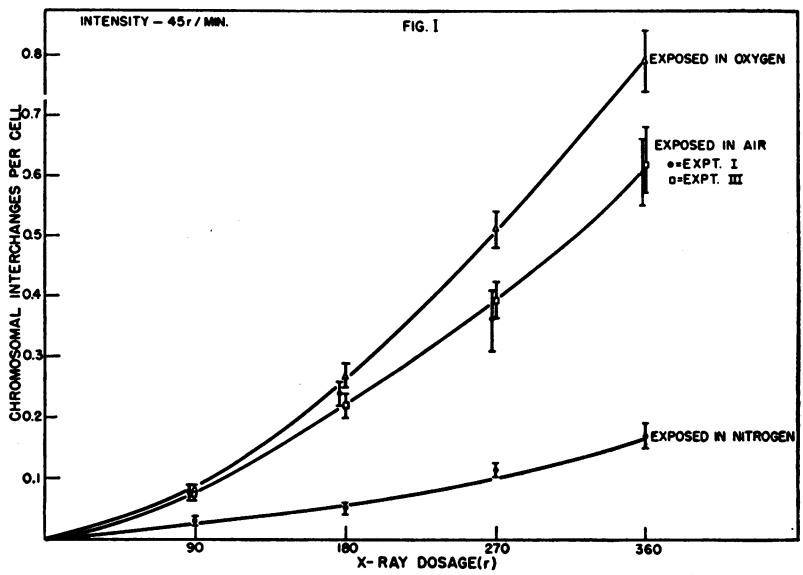

FIGURE 1

X-ray dosage curves for interchanges (dicentrics and centric rings) induced in Tradescantia microspores exposed in atmospheres of air, nitrogen and oxygen. Vertical bars indicate limits of standard errors.

in the scoring of interstitial deletions because of the small size of some of these aberration types and the more extensive data of only one investigator (H. P. R.) have been used in all except the first experiment. In general, these values are lower than those obtained in comparable scorings by the other author (N. H. G.) by approximately 20%. Despite this, the relative differences from one experiment to another are consistent in both sets of data.

It is clear from these data that the aberration frequency is strikingly reduced when exposures are made in nitrogen instead of in air. Further,

there is a significant though not as marked a difference when oxygen is used in place of air, the result in this instance being an increased aberration yield. The magnitude of these differences is very considerable, the increase in both interchanges and deletions in exposures made in oxygen as compared with nitrogen being as much as fivefold at higher dosages. These results strongly suggest that the presence of oxygen has a marked influence in increasing radiosensitivity in this material as measured by chromosomal aberration frequency.

There is no reason to believe that the observed effects can be accounted for by a modification of the timing of the cell cycle as a result of exposure

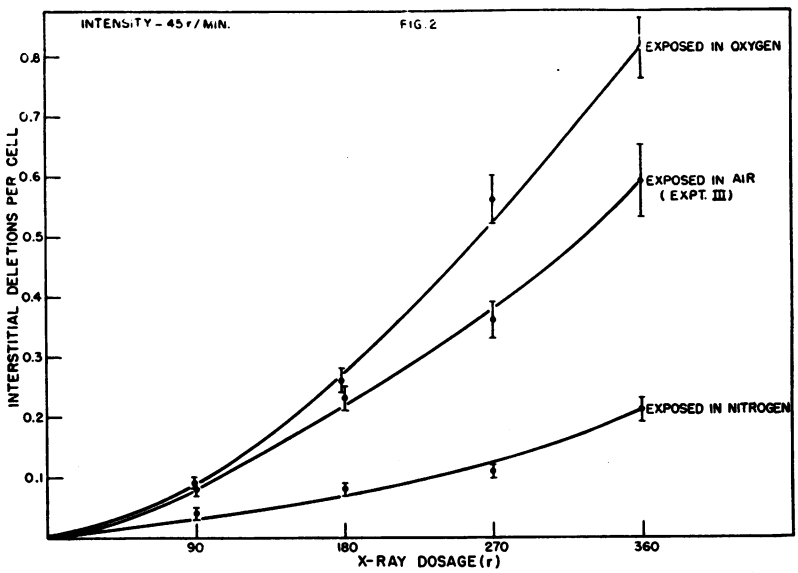

FIGURE 2

X-ray dosage curves for interstitial deletions induced in Tradescantia microspores exposed in atmospheres of air, nitrogen and oxygen. Vertical bars indicate limits of standard errors.

to the different gases such that variations in mitotic sensitivity are involved. Observations of the x-ray effects were not made until the fourth and fifth days following treatment, by which time any effect of a possible acceleration or retardation of the mitotic cycle should have little if any consequence. It has been shown by Sax[3] that there is very little change in x-ray sensitivity throughout the five- to six-day-long resting stage. Further, there is no evidence from the present data of significant differences in aberration frequencies in comparisons of four- and five-day material within individual experiments.

In order to eliminate the possibility that the presence of nitrogen rather than the absence of oxygen might be responsible for the observed decrease in radiosensitivity, further experiments were performed in which other gases

TABLE 1

FREQUENCIES OF INTERCHANGES AND INTERSTITIAL DELETIONS INDUCED IN TRADESCANTIA MICROSPORES EXPOSED TO X-RAYS IN AIR, IN NITROGEN AND IN OXYGEN ALL DOSAGES ADMINISTERED AT A CONSTANT INTENSITY OF 45 R/MIN.
(For further discussion, see text)

DOSE	CELLS SCORED	DICENTRICS	CENTRIC RINGS	TOTAL UNTERCHANGES	INTERCHANGES PER CELL	CELLS SCORED	INTERSTITIAL DELETIONS	INTERSTITIAL DELETIONS PER CELL
				EXPOSED IN AIR (EXPT. 1)				
90	500	29	11	40	0.08 ± 0.01	500	48	0.10 ± 0.01
180	276	45	20	65	0.24 ± 0.03	276	71	0.26 ± 0.03
270	150	44	10	54	0.36 ± 0.05	150	76	0.51 ± 0.06
360	200	92	29	121	0.61 ± 0.06	200	144	0.72 ± 0.06
				EXPOSED IN NITROGEN				
90	1150	23	9	32	0.03 ± 0.005	800	32	0.04 ± 0.01
180	400	15	4	19	0.05 ± 0.01	400	30	0.08 ± 0.01
270	1267	107	40	147	0.12 ± 0.01	800	90	0.11 ± 0.01
360	450	59	20	77	0.17 ± 0.02	450	93	0.21 ± 0.02
				EXPOSED IN AIR (EXPT. 3)				
90	1050	52	30	82	0.08 ± 0.01	800	65	0.08 ± 0.01
180	600	88	44	132	0.22 ± 0.02	600	139	0.23 ± 0.02
270	600	165	73	238	0.40 ± 0.03	400	143	0.36 ± 0.03
360	150	68	25	93	0.62 ± 0.06	150	88	0.59 ± 0.06
				EXPOSED IN OXYGEN				
90	800	50	15	65	0.08 ± 0.01	800	72	0.09 ± 0.01
180	900	165	77	242	0.27 ± 0.02	900	231	0.26 ± 0.02
270	500	175	78	253	0.51 ± 0.03	350	195	0.56 ± 0.04
360	300	157	80	237	0.79 ± 0.05	300	244	0.81 ± 0.05

TABLE 2

FREQUENCIES OF CHROMOSOMAL INTERCHANGES AND INTERSTITIAL DELETIONS INDUCED IN TRADESCANTIA MICROSPORES EXPOSED TO 360 R AT 45 R/MIN. IN ATMOSPHERES OF NITROGEN, ARGON AND HELIUM

TREATMENT	CELLS SCORED	DICENTRICS	CENTRIC RINGS	TOTAL INTERCHANGES	INTERCHANGES PER CELL	CELLS SCORED	INTERSTITIAL DELETIONS	INTERSTITIAL DELETIONS PER CELL
360 r in nitrogen	500	96	36	132	0.26 ± 0.02	150	56	0.19 ± 0.05
360 r in argon	500	130	50	180	0.36 ± 0.03	300	164	0.27 ± 0.04
360 r in helium	300	38	14	52	0.17 ± 0.02	150	40	0.13 ± 0.04

were substituted for nitrogen. The results of such an experiment are presented in table 2. In this instance, comparative exposures were made at a single dose, 360 r at 45 r/min., in nitrogen, in helium and in argon. It will

be noted that helium is even more effective than nitrogen in this experiment in reducing the frequency of interchanges and deletions, the values obtained in this gas being as low as or lower than those found in the nitrogen exposure at 360 r in the first series of experiments. The value for interchanges in nitrogen is somewhat higher in this experiment than in the previous one, but is still markedly less than that obtained in air. The aberration frequencies in argon, although much lower than those in air, are higher than those obtained in nitrogen or helium. The reason for this difference is not apparent but is probably due to uncontrolled variables in the experimental techniques. It is clear from this experiment, however, that the reduced aberration frequencies as compared with air cannot be attributed to the presence of a particular gas, such as nitrogen, but must result from the absence of oxygen.

As yet no exposures in atmospheres of oxygen of known intermediate percentage composition, such as 5 or 10%, have been attempted. However, some experiments were performed in which the oxygen percentage was probably close to these intermediate values. These results were inadvertently obtained when an undiscovered air leak was present in the lucite exposure box resulting in a mixture of air and the gas being introduced. The interchange frequencies per cell at 360 r (45 r/min.) under these conditions were all intermediate between those obtained in exposures made in air and in the pure gases. These values were as follows: nitrogen, 0.43 $\pm$ 0.03; helium, 0.54 $\pm$ 0.03; argon, 0.52 $\pm$ 0.03 and 0.44 $\pm$ 0.03 (different experiments). The frequencies of interstitial deletions were also intermediate between those obtained in air and in the pure gases. The values (H. P. R. only) were: nitrogen, 0.53 $\pm$ 0.04; helium, 0.41 $\pm$ 0.03; argon, 0.42 $\pm$ 0.04 and 0.32 $\pm$ 0.03. It thus appears clear that the aberration frequency increases as the percentage of oxygen increases, but it is not yet possible to make a quantitative evaluation of this relation.

In attempting to interpret the mechanism by which oxygen increases the radiosensitivity of Tradescantia chromosomes, it is immediately necessary to decide whether this effect results from an increase in the initial frequency of x-ray-induced breaks or from an effect on the reunion of broken ends, such that recombination is favored over restitution. It is of course possible that both of these effects may be present. A preliminary analysis of the dosage curves for interchanges at a constant intensity of 45 r/min. utilizing Lea's[4] formulation for the recovery time, τ,[7] suggests that the oxygen effect may be exerted principally by way of the recovery mechanism. However, it appears necessary to perform further exposures at higher and lower intensities in order to obtain more data on this point. Additional evidence is also being sought by means of another experimental approach. Previous investigations of a number of workers have indicated that in Tradescantia x-ray-induced chromosome breaks may remain open (i.e., available for participation in interchanges) for a considerable period of time. The

average restitution time as calculated by Lea[4] is about four minutes. Thus it should be possible, if oxygen exerts its effect on the recovery mechanism so as to favor recombination, to increase the yield of rearrangements by transferring inflorescences irradiated at high intensity in nitrogen to oxygen as soon as possible after exposure. An initial attempt to perform such an experiment has not been conclusive. Buds were evacuated and nitrogen admitted in the usual fashion. The buds were then exposed to 300 r at 200 r/min. in the lucite box in air in order to facilitate removing half of the inflorescences to oxygen as rapidly as possible at the end of the irradiation. This transfer required approximately one minute. It was found that the frequency of interchanges was the same in both the control half (remaining in the lucite chamber) and in the experimental half (transferred to oxygen). The actual value obtained in the control group not transferred to oxygen was higher than expected as compared to comparable exposures in air at this intensity, however, and may indicate that the replacement of air by nitrogen in the buds alone is not sufficient, but that it is necessary to make the exposure in an atmosphere devoid of oxygen as well. This problem is being investigated further.

Summary.—Experiments have been performed which indicate that oxygen has a marked effect in increasing the radiosensitivity of Tradescantia chromosomes. Comparative exposures of inflorescences to x-rays were made in air, in nitrogen and in oxygen. A cytological analysis was then made of chromosomal rearrangements (interchanges and interstitial deletions) in microspores at the four- to five-day interval following irradiation. The frequency of aberrations was strikingly reduced when nitrogen replaced air and increased when oxygen replaced air. The magnitude of this effect is very great, the increase in aberration frequency in exposures made in oxygen as compared with nitrogen being as much as fivefold at higher doses. Aberration frequencies were also reduced in additional exposures made in other gases such as helium and argon, demonstrating that the availability of oxygen is a very important factor in the production of chromosomal rearrangements by x-rays. It is not possible on the present evidence to decide whether this effect of oxygen is exerted on the initial breakage mechanism or on the recovery process.

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[7] The authors would like to express their appreciation to Dr. W. A. Arnold of this laboratory for making these calculations.