PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES

Volume 36 June 15, 1950 Number 6

*STUDIES ON THE MECHANISM OF THE OXYGEN EFFECT ON THE RADIOSENSITIVITY OF TRADESCANTIA CHROMOSOMES**

By Norman H. Giles, Jr., and Herbert Parkes Riley†

Biology Division, Oak Ridge National Laboratory

Communicated by Karl Sax, April 18, 1950

Previous experiments (Giles and Riley[1]) have demonstrated that the radiosensitivity of Tradescantia chromosomes, as measured by the occurrence of x-ray-induced aberrations in microspores, is markedly influenced by the amount of oxygen present. The frequencies of both interchanges and interstitial deletions observed four to five days following treatment are reduced if inflorescences are irradiated in gases such as nitrogen and helium and increased if exposures are made in pure oxygen instead of in air. On the basis of these experiments it was not possible to decide whether this effect of oxygen is exerted by way of the initial breakage mechanism, such that more breaks are produced by x-rays in the presence of oxygen, or whether the effect is on the recovery process, such that new reunions of broken ends are favored over restitutions. The present paper will discuss experiments performed to investigate certain aspects of this problem. Additional data will also be presented on the relation between aberration frequency and the percentage of oxygen present at the time of irradiation.

Experimental Methods.—Inflorescences of *Tradescantia paludosa* Anders. and Woodson, clone 5 (unless otherwise noted) of Sax were used. Aceto-carmine smear preparations of microspores at the first postmeiotic mitosis were made on the fourth and fifth days following irradiation and slides were scored for chromosomal aberrations—interchanges (dicentrics and centric rings) and interstitial deletions. In general, 50 or 100 cells from three to eight slides (each from a separate inflorescence) were scored and standard errors were calculated as previously. The same x-ray source was utilized—a Coolidge self-rectifying tube with tungsten target, operated at 250 kv and 15 ma. The inherent filtration was equivalent to 3 mm. of aluminum.

Irradiations were carried out as in earlier experiments in a lucite exposure chamber. However, in order to insure a more adequate control of the gas in the chamber and to facilitate a rapid removal or introduction of gas the experimental apparatus was redesigned. The new exposure chamber was placed directly in the x-ray machine and attached by pressure tubing and appropriate stopcock arrangements to a vacuum pump, a gas cylinder, and a mercury manometer. With this apparatus, all evacuations and introductions of gases could be performed directly with the inflorescences inside the exposure chamber. Evidence will be presented that the preexposure evacuations in a suction flask, as carried out in the earlier experiments, are unnecessary. Further, it is possible with this equipment to irradiate in a vacuum or under pressure and to introduce or remove gases during the period of irradiation. The presence of a manometer makes it possible to reproduce evacuation conditions and to detect possible unexpected changes of pressure in the system.

TABLE 1

COMPARATIVE EFFECTS OF VARIOUS PRETREATMENTS AND EXPOSURE-CHAMBER CONDITIONS ON THE FREQUENCY OF X-RAY INDUCED CHROMOSOMAL REARRANGEMENTS IN TRADESCANTIA MICROSPORES

400 r AT 50 r/MIN.

PRETREATMENT	EXPOSURE CONDITIONS	NO. CELLS	INTERCHANGES	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS	I. D. PER CELL
Buds preevacuated and helium admitted	Helium in chamber	226	55	0.24 ± 0.03	51	0.23 ± 0.03
Buds preevacuated and helium admitted	Air in chamber	400	289	0.72 ± 0.04	323	0.81 ± 0.05
None	Helium in chamber	300	73	0.24 ± 0.03	72	0.24 ± 0.03

Results and Discussion.—A preliminary series of experiments was performed to determine whether the preexposure evacuations in a suction flask, as carried out in earlier experiments, were necessary to remove air enclosed around the anthers by the sepals and petals of the buds. For these tests two sets of buds were evacuated five times in a flask and helium permitted to diffuse in before irradiation. One set was placed in the exposure chamber (the original chamber was used), which was then evacuated and helium admitted; the other set was placed in the exposure chamber in air. The third set of buds was not preevacuated, but placed directly in the exposure chamber, which was then evacuated and helium admitted. All three sets received 400 r at 50 r/min. (table 1). It is clear that the pretreatment has no effect on the frequency of aberrations, but rather that the gas in which the exposure is made is the important factor. Evidently evacuation in the exposure chamber is sufficient to effect gas exchange in the buds. On the basis of these results, preevacuation of buds with a water pump was discontinued. Further, with the new chamber

and a vacuum pump it was possible to evacuate directly to considerably lower pressures than in the earlier experiments.

In the original experiments comparative exposures were made at only three oxygen levels—in nitrogen or helium (no oxygen), in air (ca. 21% oxygen), and in pure oxygen. It seemed of considerable interest to obtain further data in order to determine the quantitative relation between aberration frequency and percentage of oxygen during irradiation. Consequently, two separate experiments were carried out (in one clone 5 was used, in the other, clone 3 of Sax) in which exposures to a single x-ray dose—400 r at 50 r/min.—were made with seven different percentages of oxygen in the lucite chamber. The oxygen percentages were as follows:

TABLE 2

EFFECT OF VARIOUS PERCENTAGES OF OXYGEN ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN TRADESCANTIA MICROSPORES

ALL X-RAY EXPOSURES OF 400 r AT 50 r/MIN.

OXYGEN PERCENTAGE	CLONE USED	NO. CELLS	NO. INTERCHANGES	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS	I. D. PER CELL
0	Clone 3	450	120	0.27 ± 0.02	104	0.23 ± 0.02
	Clone 5	400	113	0.28 ± 0.03	88	0.22 ± 0.02
2	Clone 3	284	73	0.26 ± 0.03	95	0.33 ± 0.03
	Clone 5	315	82	0.26 ± 0.03	77	0.24 ± 0.03
10	Clone 3	425	304	0.72 ± 0.04	305	0.72 ± 0.04
	Clone 5	200	152	0.76 ± 0.06	162	0.81 ± 0.06
21 (Air)	Clone 3	350	322	0.92 ± 0.05	392	1.12 ± 0.06
	Clone 5	150	118	0.79 ± 0.07	116	0.77 ± 0.07
60	Clone 3	303	283	0.93 ± 0.06	364	1.20 ± 0.06
	Clone 5	200	181	0.91 ± 0.07	196	0.98 ± 0.07
100	Clone 3	250	249	1.00 ± 0.06	318	1.27 ± 0.07
	Clone 5	150	148	0.99 ± 0.08	159	1.06 ± 0.08
100 (at an absolute pressure of 1500 mm. Hg)	Clone 3	225	245	1.09 ± 0.07	279	1.24 ± 0.07

0% (irradiation in pure—99.8%—helium); 2% (+98% helium); 10% (+90% helium); 21% (air); 60% (+40% helium); 99.5% (pure oxygen from a commercial cylinder); and pure oxygen at an absolute pressure of 1500 mm. of mercury (approximately 760 mm. above normal atmospheric pressure at Oak Ridge). Before each exposure, inflorescences were placed in the chamber, which was then evacuated to approximately 1 to 2 mm. of mercury, and the particular gas or gas mixture admitted. This procedure was repeated five times. Following irradiation, the inflorescences remained in the chamber for approximately ten minutes in the same gas and were then removed to air. The results of these experiments are presented in table 2 and figure 1. There is good agreement between the two

experiments except for the two points in air, where, for some unexplained reason, the values obtained for clone 3 are considerably higher than expected. The data indicate that there is a very rapid rise in aberration frequency between 2 and 20% oxygen, after which a gradual increase apparently occurs. The significance of the fact that essentially the same aberration frequencies were obtained in exposures made in pure (99.8%) helium and 2% oxygen (+98% helium) is not yet clear. This may mean that not all of the dissolved air is removed from the tissues by the evacuation procedure. It is also desirable to check further the accuracy of the reported percentage of oxygen in the gas mixture used (obtained from a

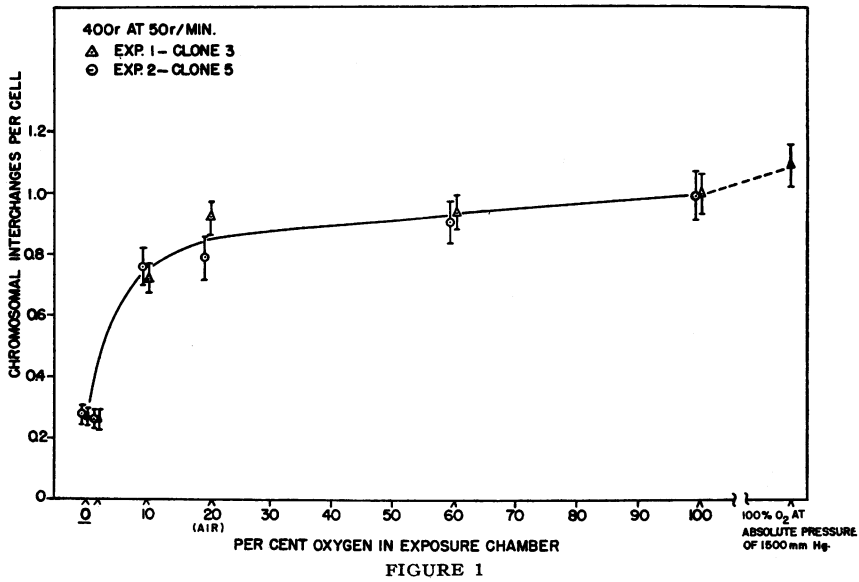

FIGURE 1

Relation between percentage of oxygen in exposure chamber and frequency of chromosomal interchanges per cell in Tradescantia microspores. All exposure to one x-ray dosage—400 r at 50 r/min. Two separate experiments, one with clone 3 and one with clone 5, as indicated.

commercial source). The general problem of the effect of oxygen during irradiation at low oxygen tensions is being investigated further.

The major problem requiring further investigation was concerned with the mechanism of the oxygen effect in increasing aberration frequencies—whether this effect resulted from a higher initial production of chromosome breaks, or from a relative increase in new reunions as opposed to restitutions of broken ends during the recovery process. It is clear from the earlier studies of Sax,[2] Marinelli, Nebel, Giles and Charles,[3] and Lea and Catche-

side,[4] that in Tradescantia there is an appreciable time interval between the production of a break and its disappearance, either by restitution or new reunion (interchange). The average time of restitution for the majority of the breaks has been estimated by Lea[5] to be about four minutes. Thus it would appear to be experimentally feasible to determine whether the oxygen effect is on initial breakage or on recovery if these two processes can be made to take place under different conditions with respect to the presence or absence of oxygen. The following series of comparative exposures (all at a single constant dosage of 300 r at 300 r/min.) was

TABLE 3

EXPERIMENTS ON THE MECHANISM OF THE EFFECT OF OXYGEN IN INCREASING THE RADIOSENSITIVITY OF TRADESCANTIA CHROMOSOMES. FOR FURTHER DISCUSSION, SEE TEXT.

ALL EXPOSURES: 300 r AT 300 r/MIN.

SERIES NO.	PRE-TREATMENT CONDITIONS	EXPOSURE CONDITIONS	POSTTREATMENT CONDITIONS	NO. CELLS	INTERCHANGES PER CELL	INTERSTITIAL DELETIONS PER CELL
1	Buds in vacuum	Vacuum	Vacuum—10 min.	880	0.12 ± 0.01	0.11 ± 0.01
2	Buds in vacuum	Vacuum	Oxygen introduced (within 3 secs.) to 1500 mm. Hg—10 min.	700	0.09 ± 0.01	0.10 ± 0.01
3	Buds in oxygen	Oxygen at 1500 mm. Hg	Oxygen at 1500 mm. Hg—10 min.	150	0.70 ± 0.07	0.83 ± 0.07
4	Buds in oxygen	Oxygen at 1500 mm. Hg	Evacuation (within 25 sec.); vacuum 10 min.	200	0.72 ± 0.06	0.85 ± 0.07
5	Buds in vacuum	1st 30 sec., vacuum. 2nd 30 sec., oxygen introduced (within 3 sec.) to 1500 mm. Hg	Evacuation (within 25 sec.); vacuum —10 min.	350	0.39 ± 0.03	0.50 ± 0.04
6	Buds in oxygen	1st 30 sec., oxygen at 1500 mm. Hg. 2nd 30 sec., evacuated (within 25 sec.) to 1 to 2 mm. Hg	Oxygen introduced (within 3 sec.) to 1500 mm. Hg—10 min.	518	0.61 ± 0.03	0.59 ± 0.03

accordingly carried out. The exposure conditions about to be described are summarized in table 3. In series 1 and 2 buds were evacuated for five minutes at 1 to 2 mm. of mercury and irradiated in vacuum. Series 1 was maintained in vacuum in the exposure chamber for ten minutes following irradiation. In series 2 pure oxygen was introduced into the exposure chamber to an absolute pressure of 1500 mm. of mercury immediately following irradiation. The introduction of oxygen to this pressure was effected within three seconds following cessation of the irradiation and the buds were maintained in oxygen for ten minutes. In series 3 and 4

buds were placed in oxygen (by the usual procedure for evacuation and introduction of gas) at an absolute pressure of 1500 mm. of mercury and irradiated in oxygen. Series 3 was maintained in oxygen for ten minutes after irradiation. In series 4 the chamber was evacuated immediately following irradiation. This evacuation to 1 to 2 mm. of mercury was accomplished within 25 seconds following the cessation of the irradiation and the vacuum was maintained for ten minutes. In series 5 buds were evacuated as in series 1 and 2 and irradiation was commenced with the buds in a vacuum. At the end of 30 seconds of exposure, oxygen was introduced into the chamber, without interrupting the irradiation, to an absolute pressure of 1500 mm. of mercury (within three seconds) and after the cessation of the total irradiation time of 60 seconds, the chamber was immediately evacuated to 1 to 2 mm. of mercury (within 25 seconds) and the buds kept in vacuum for ten minutes. In series 6 buds were placed in oxygen as in series 3 and 4 and irradiation was commenced with the buds in oxygen. At the end of 30 seconds of exposure the chamber was evacuated, without interrupting the irradiation, to 1 to 2 mm. of mercury (within 25 seconds) and after the cessation of the total irradiation time of 60 seconds oxygen was reintroduced to an absolute pressure of 1500 mm. of mercury (within three seconds). The exposure of 300 r/min. for one minute was selected to make the total time of exposure as short as feasible compared to the average time for restitution, since restitution takes place during the period of irradiation also. The data obtained from these exposures are presented in table 3.

It is clear from the first comparison (series 1 and 2) that the addition of oxygen immediately after irradiation does not increase the frequency of aberrations. Such an increase would be expected if there were an effect of oxygen on the reunion process. Nor does the removal of oxygen (series 3 and 4) after irradiation result in a lower aberration frequency. In both comparisons the observed aberration frequency apparently depends on the presence or absence of oxygen at the time of irradiation. The additional experiments were included to test this point further and also to exclude the possibility that the addition or removal of oxygen (to or from the cells themselves) after irradiation was not accomplished rapidly enough to detect an effect on the reunion process if such an effect existed. In series 5, it is evident that the addition of oxygen during irradiation results in a marked increase in aberration frequency. There is apparently an almost immediate entrance of a considerable amount of oxygen into the cells of the anthers at the beginning of the final 30 seconds of irradiation (the period of three seconds indicated is the time required to introduce oxygen to a pressure of 1500 mm. of mercury; considerably less time is probably required for the introduction of sufficient oxygen to produce an almost maximal increase in aberration frequency). In series 6 there is an appreciable decrease in aberration frequency accompanying the removal of

oxygen during the last 30 seconds of radiation. The fact that this decrease is not as marked as the increase noted on the addition of oxygen in series 5 appears to be quite reasonable, since a considerably longer period is required to evacuate the chamber than to introduce oxygen.

On the basis of the data which have been presented it is clear that the postirradiation presence or absence of oxygen has no effect on the recovery process. It seems reasonable to conclude also that there is no effect of oxygen on the recovery process occurring during irradiation, which in these experiments occupies a relatively small fraction of the total recovery period. Pretreatment of buds in the presence or absence of oxygen has no effect; oxygen must be present during the actual X-ray exposure to produce an increase in aberration frequency. Furthermore, the experiments in which oxygen is introduced during irradiation indicate that its effect is immediate. It thus appears that the effect of oxygen must be exerted on the breakage mechanism. However, it seems probable that this effect is actually an indirect one. The most likely hypothesis seems to be that when dissolved oxygen is present during irradiation in the largely aqueous medium in cells, some substance resulting from the radiodecomposition of water containing oxygen is formed which causes an increased aberration frequency. On the basis of their experiments with *Vicia faba*, Thoday and Reed[6] suggest that this substance may be hydrogen peroxide. This possibility is supported by the results obtained in the present experiments relating aberration frequency to percentage of oxygen at the time of irradiation. This relation (fig. 1) is generally similar to that for the yield of hydrogen peroxide when water containing increasing concentrations of oxygen is subjected to X rays (Bonet-Maury and Lefort[7]). If it is assumed that hydrogen peroxide is in fact the substance indirectly responsible for the increased aberration frequency obtained in oxygen, it appears likely that such an increase would result from a higher frequency of initial breakage, rather than from an effect on the recovery process. This conclusion is supported by the observations of Baker and Sgourakis[8] that oxygen increases the yield of X-ray induced sex-linked lethal mutations in *Drosophila*, where there is no evidence that a recovery process is involved. However, the possibility cannot yet be excluded that in Tradescantia an intermediate radiation product such as hydrogen peroxide might produce an effect by modifying the behavior of broken ends, themselves produced by direct radiation action, and thus influence the restitution process.

Summary.—Further experiments have been performed on the effect of oxygen in increasing the radiosensitivity of Tradescantia microspore chromosomes. Exposures of inflorescences to a single constant x-ray dose, but in atmospheres containing seven different percentages of oxygen indicate that there is a rapid rise in aberration frequency between 2 and 21% oxygen, with a gradual increase thereafter. Further studies are being made to clarify the effect of oxygen at levels between zero and two

per cent. Other experiments have been performed to determine whether the oxygen effect is exerted by way of the initial breakage mechanism or on the reunion process. These consisted of comparative exposures to a single dose of 300 r in one minute of inflorescences in a vacuum or in oxygen with the addition or removal of oxygen either immediately after or during part of the irradiation period. In addition, buds were pretreated in the presence and absence of oxygen before exposure to X rays. These experiments show that the presence of oxygen during the actual exposure to X rays rather than during the pre- or postirradiation period is the important factor, thus indicating that oxygen alone does not influence the recovery process. It seems likely that the oxygen effect is an indirect one, resulting from the production during irradiation in oxygen of some substance such as hydrogen peroxide. Although it appears probable that the effect of such a substance on aberration frequency would result from an increased production of chromosome breaks, the alternative possibility, that such a substance might modify the restitution process, cannot yet be excluded.

Acknowledgment.—The authors wish to acknowledge the assistance of Lucille McMichael Fairchild in certain of these experiments.

* This work was done under Contract No. W-7405-Eng-26 for the Atomic Energy Commission, Oak Ridge, Tennessee.

† Present address: Department of Botany, University of Kentucky, Lexington, Ky.

[1] Giles, N. H., Jr., and Riley, H. P., these PROCEEDINGS, **35**, 640 (1949).

[2] Sax, Karl, *Ibid.*, **25**, 225 (1939).

[3] Marinelli, L. D., Nebel, B. R., Giles, N. H., Jr., and Charles, D. R., *Am. J. Botany*, **29**, 866 (1942).

[4] Lea, D. E., and Catcheside, D. G., *J. Genetics*, **44**, 216 (1942).

[5] Lea, D. E., *Actions of Radiations on Living Cells*, Cambridge University Press, Cambridge, England, 1945.

[6] Thoday, J. M., and J. Reed, *Nature*, **163**, 133 (1949).

[7] Bonet-Maury, P., and M. Lefort, *Nature*, **162**, 381 (1948).

[8] Baker, W. K., and E. Sgourakis, these PROCEEDINGS, **36**, 176 (1950).

THE ORIGIN AND BEHAVIOR OF MUTABLE LOCI IN MAIZE

BY BARBARA McCLINTOCK

DEPARTMENT OF GENETICS, CARNEGIE INSTITUTION, COLD SPRING HARBOR, NEW YORK

Communicated April 8, 1950

In the course of an experiment designed to reveal the genic composition of the short arm of chromosome 9, a phenomenon of rare occurrence (or recognition) in maize began to appear with remarkably high frequencies in the cultures. The terms mutable genes, unstable genes, variegation,