MODIFICATION OF CHROMOSOMAL ABERRATION YIELD BY POSTIRRADIATION TREATMENT

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FOLLOWING THODAY and READ's (1947) discovery that *Vicia faba* roots suffered more chromosomal damage when X-irradiated in the presence of oxygen than in its absence, much work has been performed to elucidate the mechanism of this "oxygen effect." The aberrations observed are usually the result of two different processes that can occur, i.e., breakage of the chromosome and the subsequent rejoining of these breaks. This is because, once formed, breaks can either remain open (one-hit aberrations) or rejoin in one of two different ways—either restituting, which is rejoining in the original configuration (no aberrations), or reuniting illegitimately with other broken ends (two-hit aberrations). It was therefore obvious that modification of either of the two processes could affect the yield of aberrations obtained with a given X-ray dose. GILES and RILEY (1950) in a series of experiments with *Tradescantia* microspores, the classical material for the study of radiation-induced chromosome aberrations, found that if they added oxygen very shortly after irradiation they did not get any increased aberration yield. They concluded, therefore, that the oxygen had to be present at the time of irradiation to be effective, that the increased aberration frequency obtained in oxygen resulted from a higher frequency of initial breakage, and that the post-irradiation presence or absence of oxygen had no effect on the recovery (viz., rejoining) process.

Extensive dose fractionation studies by WOLFF and coworkers (WOLFF and ATWOOD 1954; WOLFF and LUIPPOLD 1955, 1956) on X-ray-induced chromosomal aberrations in the root tips of *Vicia faba* seeds, confirmed the conclusion of GILES and RILEY that the "oxygen effect" was caused by increased breakage. However, these latter studies designed to detect any effect on rejoining led to the rejection of the assumption that there was no effect whatsoever on the recovery (rejoining) mechanism. In fact, their results indicated a postirradiation effect of oxygen on the *time* (albeit not the *amount*) of rejoining.

Since studies on the postirradiation modification of radiation-induced genetic damage are of great theoretical importance, and also since the "oxygen effect" has already been used clinically to modify the radiation sensitivity of tumors (GRAY 1956) in human beings, it seemed necessary to reinvestigate the post-irradiation effect in *Tradescantia*. This was thought to be particularly pertinent because some of the original data of GILES and RILEY appear to be consistent with a concept derived from the *Vicia* work by WOLFF and ATWOOD (1954), that there

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are two independent effects of irradiation: one on breakage and the other on rejoining.

Other conclusions from the *Vicia* studies tested in the present experiments on *Tradescantia* are that after irradiation in the presence of oxygen, the chromosome breaks produced remain open longer than when the irradiation is performed anoxically (WOLFF and ATWOOD 1954), and that the rejoining of breaks can be inhibited by the postirradiation inhibition of cellular respiration and probably of adenosinetriphosphate (ATP) production (WOLFF and LUIPPOLD 1955, 1956).

METHODS

In order to test whether or not a postirradiation effect of oxygen on aberration production existed, we decided to use the same method as previously, i.e., a dosage fractionation experiment. The rationale for this is as follows: for exchange formation to occur, two breaks must be open concurrently and be close to each other. If one considers a small volume within the nucleus and assumes that if two breaks are within this volume they will rejoin to form an exchange, then there will be a probability, p , that a break will lie within this volume and a probability, $p \times p$ or p^2 , that two breaks will simultaneously lie within the volume and thus form an exchange. It is assumed that p is proportional to the number of breaks induced and thus to the dose. However, as calculated by LEA (1947), 95 percent of the breaks formed reconstitute. If the radiation is fractionated into two doses, so that reconstitution of the breaks produced by the first dose occurs before administration of the second dose, then the number of exchanges observed will be the sum of those produced by each of the doses. However, if the second dose is given before the first group of breaks reconstitute, then these breaks will be present concurrently with the second group and the exchanges will increase as the square of the total dose. By the inhibition of cellular respiration between two doses administered to *Vicia* seeds, it was possible to keep breaks from reconstituting. The fractionation experiments herein described were performed to test whether or not there was a similar effect in *Tradescantia* microspores, especially since postirradiation anoxia had been reported to have no effect in this material.

Before the experiments could be performed, however, it was necessary to determine how long a period of time was needed to ensure rejoining of the breaks produced by the first dose. SAX *et al.* (1955) reported that the breaks induced in *Tradescantia* microspores by a dose of 75r remained open for about one hour. In *Vicia* seed, this time was found to be dose dependent (WOLFF and ATWOOD 1954) and so it was necessary to perform an intensity experiment to determine how long the breaks in *Tradescantia* microspores would remain open with our experimental procedure. An intensity experiment is similar to a fractionation experiment in that as intensity of radiation decreases, if reconstitution occurs, fewer breaks will be open concurrently and the number of exchanges induced by a given dose will decrease. If the intensity is such that the total dose is administered before any appreciable number of the breaks can reconstitute, there will be no such decrease.

Thus the length of the plateau on an intensity curve is a measure of the time breaks remain open before restituting.

All experiments were performed on inflorescences of *Tradescantia paludosa*, (clone 3 of SAX). Four or five days after irradiation, acetocarmine smear preparations were made of microspores at the first postmeiotic mitosis. The slides were scored for two-hit chromosomal aberrations (dicentrics and rings). The X-ray source was a G.E. Maxitron operated at 250 kvp with 3 mm of Al filtration added. For treatment with vacuum or a gas, the inflorescences were placed in lucite chambers. The chambers were evacuated with a vacuum pump for 3–5 minutes before gas was introduced. In those experiments with 95 percent CO + 5 percent O₂, after evacuation, oxygen was admitted to bring the pressure up to 1/20 of an atmosphere. Then CO was added until the pressure was normal. A sodium arc lamp at a distance of 6 cm (720 ft-c as measured by a Weston Illumination meter 756 Viscor filter) was utilized to effect light reversibility of the cytochrome oxidase-CO complex. For those experiments requiring irradiation in the gas, the chamber itself was placed in the X-ray beam. All dosimetry was done with a Victoreen Radocon.

RESULTS AND DISCUSSION

The intensity curve presented in Figure 1 indicates that when the inflorescences are exposed to 200r, there is no apparent decrease in aberration yield until the irradiation was spread over 40 minutes; that is, all the breaks remained open and capable of forming an exchange for at least 20 minutes before some of them started

FIGURE 1.—The effect of varying the intensity on the production of two-hit aberrations induced by radiation in air and in nitrogen.

to close. When the irradiation was performed in nitrogen, a double effect was noted: (1) fewer breaks, and consequently fewer aberrations, were formed than in air; and (2) the breaks induced rejoined much faster than those induced in air. By the initial slope of the nitrogen curve, it may be concluded that breaks produced in nitrogen remained opened for a maximum of about 2.5 minutes. The general phenomenon of two independent effects of the radiation, one on breakage and one on rejoining, has already been described for *Vicia* (WOLFF and ATWOOD 1954) and for *Trillium* (DESCHNER and SPARROW 1955). The same phenomenon seems therefore to apply in *Tradescantia*. However, it must be noted that the time scale is different in *Tradescantia* since in this genus nitrogen-induced breaks rejoin in a very short time. This difference had already been apparent from the studies on postirradiation centrifugation of *Tradescantia* inflorescences. There it was noted for both chromosome (WOLFF and VON BORSTEL 1955) and chromatid (ANDERSON 1955) aberrations that the breaks produced in nitrogen rejoined too quickly to be moved apart by centrifugation. The same phenomenon of very rapid rejoining of breaks induced anoxically has also been observed by FETNER (1956). Although he states that the breaks remained open for long periods of time, his data from dosage fractionation studies show that even with zero time between doses, the aberration yield has approached the base line and is simply the sum of the two-hit aberrations produced by each of the half doses. This indicates that the breaks induced under conditions of partial anoxia, instead of staying open for a long time, have already rejoined before the four minutes required for the completion of the dose.

Our intensity experiments demonstrated that, unlike *Vicia*, the *Tradescantia* breaks induced in nitrogen closed too quickly to allow the easy administration of postirradiation treatments. The breaks induced in air, however, seemed to stay open for the relatively long time of at least 20 minutes. At a lower dose we expected this time to be even less (WOLFF and ATWOOD 1954) but the fractionation experiment in Table 1 shows that the breaks induced by 150r rejoin continuously over a period of about 40 minutes, after which one simply sees an aberration yield

TABLE 1

Effect of varying fractionation time on aberration yield induced by 300r

Dose I (r) in air 100r/min	Interval (min)	Dose II (r) in air 100r/min	No. 2-hit aberrations	
			No. cells scored	2-hit aberrations per 100 cells
0	..	...	1/200	0.5
150	0	...	49/300	16.3±2.3
300	0	...	157/300	52.3±4.2
150	5	150	148/300	49.3±4.1
150	10	150	144/300	48.0±4.0
150	20	150	119/300	39.7±3.6
150	40	150	116/300	38.7±3.6
150	80	150	102/300	34.0±3.4
150	120	150	93/300	31.0±3.2

that does not differ significantly from the sum of the aberrations produced by the two doses. For further dose fractionation experiments, therefore, a time of one hour was chosen as an adequate interval between the two doses to allow rejoining of the first breaks to occur before production of the second group. As in the *Vicia* experiments, immediately after the first dose, various treatments were administered to prevent rejoining until after the second dose had been given.

The results are seen in Table 2. It may be noted that in air in light the breaks induced by 150r have closed before the second dose (A). However, if after the first dose the inflorescences were placed in a vacuum in the dark, the breaks remained open (B). The interval was in the dark to prevent the formation of oxygen by photosynthesis during the course of the experiments. The experiments of WOLFF and LUIPPOLD (1955), have shown that for *Vicia* only very small amounts of oxygen are necessary to allow rejoining to occur, and CONGER (unpublished data) found that if *Tradescantia* microspores were irradiated in nitrogen in the dark, the breaks instead of closing would remain open for long times. Thus it seemed important to prevent the plants from producing their own oxygen. If between the two doses the inflorescences were subjected to a vacuum for only three minutes and then allowed to remain in nitrogen for the hour, again the breaks remained open and gave the same aberration yield as with a dose of 300r without any fractionation (C). If, however, for a control the plants were placed in air in the dark after the vacuum treatment, the breaks rejoined in the interval (D). It was also found that placing the plants in 95 percent CO and 5 percent O₂ after vacuum treatment kept the breaks open if it were done in the dark but not if done in the light (E and F). In experiment F, it should be noted that the interval between doses was increased to 75 minutes. This was because experiments performed at about the same time indicated that even when the interval was spent in air in light, incomplete rejoining occurred at the time of one hour.

These experiments are consistent with the previous *Vicia* experiments on post-irradiation modification of aberration yield. The one thing that all the above treatments have in common is that they inhibit cellular respiration. The light reversibility of the CO effect, in particular, indicates that cytochrome oxidase activity is necessary for the breaks to rejoin.

It may be noted that each half-dose alone results in the same total aberration yield, whether or not postirradiation treatments influence the breaks to remain open longer than they would normally. This effect has also been noticed in *Vicia* (WOLFF 1957) and indicates that only the *time* for rejoining and not the *amount* has been affected. Therefore the fact that GILES and RILEY (1950) did not observe an increase in aberration yield when they changed the gas after a single dose of radiation does not necessarily imply the lack of an effect on rejoining. To the contrary, the experiments reported in this paper indicate that there is a post-irradiation effect of oxygen on the rejoining of chromosome breaks in *Tradescantia*. It must be noted, however, that this latter effect of oxygen is to allow cellular respiration to occur and thus to allow the rejoining of breaks. The post-irradiation presence of oxygen then decreases the aberration yield (if a second

TABLE 2

The effect of postirradiation treatments on the rejoining of chromosome breaks in Tradescantia

Dose I (r) in air 200r/min	Treatment for 1-hr interval	Dose II (r) in air 200r/min	Number 2-hit aberrations		2-hit aberrations per 100 cells	Expected yield if Dose I and Dose II are additive
			Number cells scored			
			Expt. 1	Expt. 2		
A						
0	Air in light	0	0/150		0.0	...
150	Air in light	0	17/116	51/350	14.6±1.8	...
150	Air in light	150	81/237	103/300	34.2±2.5	29.2
B						
150	Vacuum in dark	0	13/100	50/300	15.7±2.0	...
0	Vacuum in dark	150	22/150	43/300	14.5±1.8	...
150	Vacuum in dark	150	75/150	124/250	49.7±3.5*	30.2
0	Vacuum in dark	0	1/200		0.5	...
300	Vacuum in dark	0		105/200	53.0±5.1*	...
C						
150	Vacuum 3 min then N ₂ in dark	0	63/300		21.0±2.6	...
0	Vacuum 3 min then N ₂ in dark	150	34/300		11.3±1.9	...
150	Vacuum 3 min then N ₂ in dark	150	54/300		51.3±2.4*	32.3
0	Vacuum 3 min then N ₂ in dark	0	0/150		0.0	...
D						
0	Vacuum 3 min then air in dark	0	0/150		0.0	...
150	Vacuum 3 min then air in dark	0	52/300		17.6±2.4	...
0	Vacuum 3 min then air in dark	150	35/200		17.5±3.0	...
150	Vacuum 3 min then air in dark	150	123/300		41.0±3.7	36.1
E						
150	95% CO + 5% O ₂ in dark	0	38/200		19.0±3.1	...
0	95% CO + 5% O ₂ in dark	150	52/300		17.3±2.4	...
150	95% CO + 5% O ₂ in dark	150	155/300		50.7±4.2*	36.3
150	95% CO + 5% O ₂ in light	0	46/300		15.3±2.3	...
0	95% CO + 5% O ₂ in light	150	48/250		19.2±2.8	...
150	95% CO + 5% O ₂ in light	150	132/350		37.4±3.3	34.5
F†						
150	Air in light	0	42/300		14.0±2.2	...
300	Air in light	0	135/244		55.2±4.8*	...
150	Air in light	150	105/300		35.0±3.4	28.0
150	95% CO + 5% O ₂ in light	150	106/300		35.3±3.4	28.0
150	95% CO + 5% O ₂ in dark	150	141/300		47.0±4.0*	28.0

* If the breaks induced by Dose I remain open to rejoin with those of Dose II, the aberration yield should be the same as that observed after an unfractionated dose of 300r.

† Interval 75 minutes.

dose is given) which is the opposite result from that ordinarily called "oxygen effect." We therefore believe that it is erroneous to interpret our data on the post-irradiation effect of oxygen as indicating that the "oxygen effect" of increased damage results from an effect on rejoining (BAKER 1955). We also believe that the "oxygen effect" of increased aberration yield has been correctly stated by GILES and RILEY to be an effect on the breakage of the chromosomes.

These results have been confirmed both in *Drosophila* and in *Allium*. ABRAHAMSON (1958) working with *Drosophila* oocytes found that if oxygen were present between two doses, the breaks from the first would close before the second dose was given; whereas if the flies were kept in nitrogen for the interval, then the breaks remained open (i.e., capable of forming an exchange). COHN, working with onion seeds (1956) found that CO in the dark would keep breaks open but that in the light it would not.

In *Drosophila*, however, the *amount* of rejoining as well as the *time* seemed to be affected. This was indicated by the observation that the aberration yield induced by a single dose was increased if the breaks were kept open by postirradiation treatments of nitrogen to females (ABRAHAMSON 1958) or cyanide to males (SOBELS 1957).

In *Vicia* where it is feasible to treat with solutions of inhibitors, in addition to gases, similar experiments (WOLFF and LUIPPOLD 1955) with dinitrophenol as an uncoupler of oxidative phosphorylation from respiration, have indicated that ATP is necessary for the rejoining of breaks. It has also been shown (WOLFF and LUIPPOLD 1956) that the addition of exogenous ATP stimulates rejoining.

The experimental work with *Vicia* led to the postulation (WOLFF and ATWOOD 1954; WOLFF and LUIPPOLD 1956) that radiation independently affects the number of chromosome breaks in the cell and the time that they will remain open. Oxygen present at the time of irradiation increases the amount of damage. This is reflected both in an increased number of breaks and in their remaining open longer. In the presence of ATP produced by respiration, however, recovery can occur and the breaks can rejoin. If the respiration is inhibited after irradiation (this can be done by anoxia), then little ATP is produced and the breaks cannot rejoin. This has been represented schematically in Figure 2. The presence of a "rejoining system" has been postulated to account for both the independence of the time of rejoining from the number of breaks induced and the dependence of this time on radiation dose.

The predictions of this theory, in addition to fitting the results observed in *Vicia*, *Tradescantia*, *Allium*, and *Drosophila*, also fit such diverse phenomena as the reverse "oxygen effect" of BEATTY *et al.* (1956) and the results obtained by GILES and RILEY (1950) when they changed gases during irradiation. In the former case it may be argued that after long periods in helium a state of complete anoxia results and breaks induced in *Tradescantia* remained open to rejoin with breaks induced at a later time. This then raises the aberration yield under anoxic conditions and gives a reverse oxygen effect (see SWANSON and KIHLMAN 1956). In the latter case GILES and RILEY observed that when they administered the

FIGURE 2.—Schematic representation of effects of radiation on production of X-ray induced chromosome exchanges.

first half of the dose *in vacuo* and the second half in oxygen, they obtained a lower aberration yield than when the gases were administered vice versa. This is similar to the effect noted by WOLFF and ATWOOD (1954) in *Vicia*, when the first dose was administered anoxically. It has been argued that when the first irradiation is *in vacuo*, because of less damage to the "rejoining system," the breaks induced by the first half of the dose rejoin before the second half has been finished. When the first part of the dose is administered in oxygen, the breaks induced do not rejoin until after all the breaks are present concurrently in the system. Thus the aberration yield is increased from what is observed in the opposite case. This argument can be used as an alternative explanation of the effect observed in *Tradescantia* by GILES and RILEY which they attribute to the fact that they could change from vacuum to oxygen much faster than they could change from oxygen to vacuum.

SUMMARY

In *Tradescantia* microspores, postirradiation treatments can modify the numbers of chromosome exchanges induced by X-rays. The postirradiation inhibition of respiration by anoxia or carbon monoxide in the dark prevents the rejoining of breaks and consequently allows breaks to remain open and capable of forming exchanges with breaks induced later. The results with *Tradescantia* are qualitatively similar to the results obtained with *Vicia* in that irradiation in the presence of oxygen produces more breaks than does anoxic irradiation and that for rejoining cellular respiration (the production of ATP) is required.

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