THE EFFECT OF OXYGEN ON THE PRODUCTION BY FAST NEUTRONS OF CHROMOSOMAL ABERRATIONS IN TRADESCANTIA MICROSPORES[1]

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THE studies of THODAY and READ (1947) using root-tip cells of *Vicia faba* demonstrated that oxygen has a marked effect in increasing the frequency of X-ray-induced chromosome aberrations. These investigators later showed (1949) that when alpha rays are used, the oxygen effect is much less, since the yield of aberrations with oxygen present was only slightly greater than in its absence. In experiments on the oxygen effect using Tradescantia microspore chromosomes (GILES and RILEY 1949, 1950; GILES and BEATTY 1950), a pronounced effect of oxygen on X-ray-induced rearrangements has also been demonstrated. Preliminary studies of CONGER (unpublished) further indicate that there is little or no oxygen effect when aberrations are induced in mature pollen grains (exposed in air as compared with nitrogen) by alpha rays, thus agreeing with the observations of THODAY and READ (1949). In view of these results it seemed of interest to investigate the oxygen effect when fast neutrons are used to produce aberrations in Tradescantia, since this radiation is intermediate in effectiveness with respect to chromosome break production between X-rays and alpha particles (cf. KOTVAL and GRAY 1947).

METHODS

Inflorescences of *Tradescantia paludosa* (clone 5 of SAX) were exposed to fast neutrons produced from uranium fission in the Oak Ridge nuclear reactor in a manner generally similar to that used previously (GILES and CONGER 1950). However, in order to control the composition of the atmosphere in which the microspores were irradiated, the inflorescences were placed on a thin perforated platform inside a special airtight lucite exposure chamber from which air could be removed by evacuation and into which either pure oxygen, pure helium, or pure nitrogen could be introduced. Irradiations with fast neutrons were then performed with the inflorescences in the appropriate gas inside this chamber. Both prior to and following irradiations the intensity of the fast neutron beam in n units was determined by a series of measurements utilizing a 100 r Victoreen thimble ionization chamber placed inside the lucite container in the same position as that occupied by the inflorescences. The

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average fast neutron intensity during these exposures was 2.0 n per minute. For these measurements, one of the same Victoreen thimble chambers used in previous experiments was employed. This chamber differed by only 5 percent in comparative exposures with the thimble used as the standard in earlier experiments (GILES and CONGER 1950). This same thimble chamber, or one having essentially the same correction factor, was used in the earlier X-ray exposures (GILES and RILEY 1949, 1950). The dosage for each exposure was calculated by multiplying the total exposure time in minutes by the constant intensity of 2 n per minute. Irradiations were carried out at room temperature (*ca.* 22°C).

Most of the cytological observations were made of chromosome aberration types present in cells on slides made on the fourth and fifth days following irradiation. However, more limited observations were also made of chromatid aberration types present in microspores examined 24 hours after exposure.

RESULTS AND DISCUSSION

The data on the frequencies of chromosome aberration types are presented in table 1. In figure 1 are plotted the relationships between neutron dose and frequencies of interchanges (dicentric and centric rings) in oxygen and in nitrogen, plus two points obtained in helium. To this figure have been added for comparison the results obtained in a previous experiment with fast neutrons in which the exposure was made in air (GILES and CONGER 1950). The data on chromatid aberrations, based on observations at two dosage levels only, are presented in table 2 and in figure 2.

From these data the following conclusions may be drawn: (1) In the absence of oxygen, the frequencies of all types of aberrations (in both chromo-

TABLE 1

Frequencies of chromosomal interchanges (dicentrics and centric rings) and interstitial deletions (I.D.) induced by fast neutrons in Tradescantia microspores irradiated in atmospheres of oxygen, nitrogen, or helium (Exp. ORFN-5).

Dose (n)	No. of cells	Dicentrics	Centric rings	Total	Interchanges per cell	I.D.	I.D. per cell
			Irradiated in oxygen				
5	800	99	42	141	0.18 ± 0.015	189	0.23 ± 0.017
10	700	144	65	209	0.30 ± 0.02	243	0.35 ± 0.02
20	350	159	68	227	0.65 ± 0.02	283	0.81 ± 0.05
30	350	228	84	312	0.89 ± 0.05	402	1.14 ± 0.06
			Irradiated in nitrogen				
5	700	55	19	74	0.11 ± 0.01	94	0.13 ± 0.01
10	750	98	41	139	0.185 ± 0.016	164	0.22 ± 0.02
20	500	155	56	211	0.42 ± 0.03	295	0.59 ± 0.03
30	300	137	56	193	0.64 ± 0.046	238	0.79 ± 0.05
			Irradiated in helium				
10	650	94	43	137	0.21 ± 0.02	140	0.215 ± 0.02
30	50	25	6	31	0.62 ± 0.11	44	0.88 ± 0.13

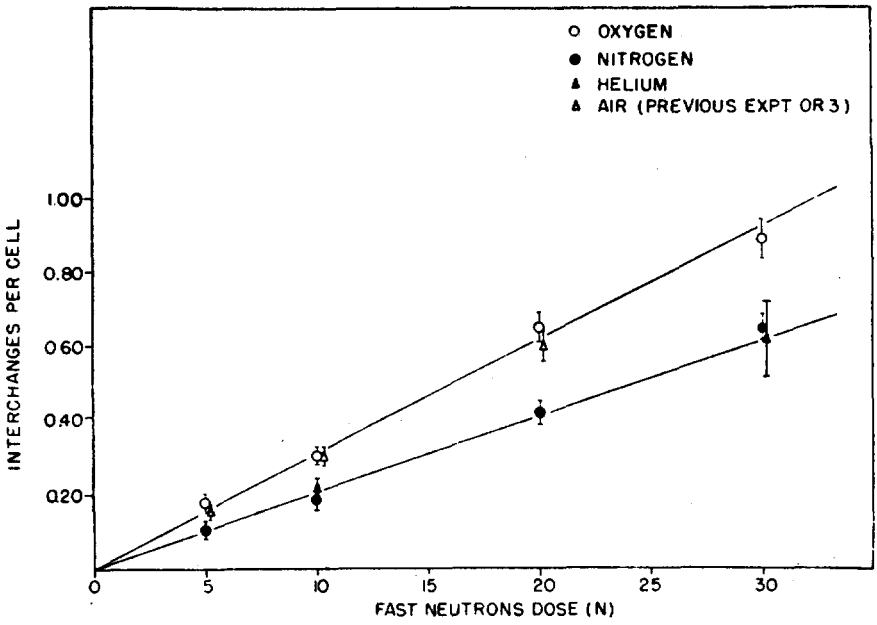

FIGURE 1.—Relation between dosage of fast neutrons and frequency of chromosomal interchanges induced in Tradescantia microspores irradiated in oxygen, air, nitrogen, or helium. The data for air were obtained in a previous experiment (ORFN-3).

some and chromatid categories) are reduced when equal dosages of neutrons are compared. (2) No significant difference is found between chromosome interchange frequencies (the only aberration type for which data are available) when neutron exposures in oxygen and in air are compared on the basis of these results and of data obtained in earlier experiments. (3) There is a linear

TABLE 2

Frequencies of chromatid aberration types induced by fast neutrons in Tradescantia microspores irradiated in atmospheres of oxygen, nitrogen, or helium (Exp. ORFN-5).

Dose (n)	No. of cells	Chromatid breaks per cell	Isochromatid breaks per cell	Exchanges per cell
		Irradiated in oxygen		
5	200	0.35 ± 0.04	0.98 ± 0.07	0.28 ± 0.04
10	200	0.77 ± 0.06	1.725 ± 0.09	0.46 ± 0.05
		Irradiated in nitrogen		
5	200	0.30 ± 0.04	0.62 ± 0.056	0.105 ± 0.02
10	200	0.58 ± 0.05	1.31 ± 0.08	0.21 ± 0.03
		Irradiated in helium		
10	200	0.58 ± 0.05	1.28 ± 0.08	0.23 ± 0.03

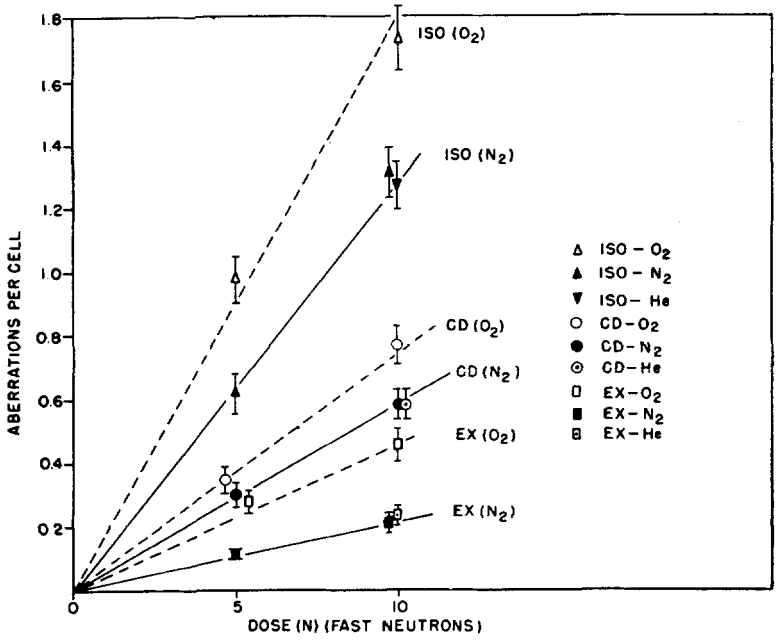

FIGURE 2.—Relation between dosage of fast neutrons and frequencies of chromatid aberration types induced in Tradescantia microspores irradiated in oxygen, in nitrogen, and in helium (Exp. ORFN-5).

relationship between neutron dose and aberration frequency for all types of aberrations (including both chromosome and chromatid interchanges) when buds are irradiated either in the presence or in the absence of oxygen.

It is clear from these results that oxygen has the effect of increasing the frequency of aberrations induced by fast neutrons. However, when a quantitative comparison is made of the present results with those obtained in experi-

TABLE 3

Comparison of the relative magnitude of the oxygen effect on chromosome aberration frequencies induced in Tradescantia by different radiations.

Radiation	Dose ratios for aberrations[3] nitrogen/oxygen				
	Chromatid breaks	Isochrom. breaks	Chromatid plus isochrom. breaks	Chromatid exchanges	Chromosome exchanges
Alpha particles	....	1.0[1]	....	1.0[1]	....
Fast neutrons	1.2	1.4	1.3	2.3	1.5
X-rays	1.4	2.6	2.0	(ca 2.5)[2]	(ca 2.5–3.5)[2]

[1]Based on preliminary data of CONGER (unpublished) obtained from irradiation of mature pollen grains in air and in nitrogen.

[2]For a further discussion of these values, see text.

[3]The dose ratio (N_2/O_2) = the ratio between a radiation dose in nitrogen and one in oxygen producing equal aberration frequencies and, consequently, is a direct measure of any effect of oxygen in increasing aberration production.

ments with X-rays (GILES and RILEY 1949, 1950; GILES and BEATTY 1950; RILEY, GILES and BEATTY 1952), it is apparent that the magnitude of the oxygen effect with fast neutrons is considerably less than it is with X-rays.

The most direct and satisfactory comparisons can be made utilizing aberrations exhibiting linear relationships with dose for both X-rays and neutrons when irradiated in oxygen or in its absence. These are the chromatid and isochromatid break types. The individual as well as the combined dose ratios for chromatid and isochromatid breaks for both X-rays and fast neutrons are shown in table 3. The most reliable cytological observations are probably for isochromatid types, and these data can also be compared directly with those of CONGER (unpublished) for alpha particles. It appears that the relation of type of radiation to the magnitude of the oxygen effect is, in increasing order, alpha particles (none), neutrons, and X-rays.

Comparison of exchange break aberrations are not as easy to make since these types show a linear relationship with dose for neutrons, but approach a dosage-square relationship with X-rays (depending on the intensity of the radiation). Unless the exponents of the comparative X-ray dosage curves obtained in oxygen and in nitrogen are equal, the value of the dose ratio (the ratio of doses which produce equal effects) will depend on the dose. In the case of X-ray-induced chromosome interchanges the evidence indicates that the exponents are different (GILES and RILEY 1950). However, a rough comparison over the X-ray-dose range used can be made and this gives nitrogen-oxygen dose-ratio values ranging from *ca.* 2.5 to *ca.* 3.5. When these values are compared with the fast neutron nitrogen-oxygen dose ratio for interchanges, obtained over a comparable range in terms of biological effect (aberration frequency per cell), again the X-ray values are higher than the neutron value of 1.5. Similar comparisons can be made for chromatid exchanges. Here the exponents of the curves obtained in oxygen and in nitrogen turned out to be essentially the same (RILEY, GILES and BEATTY 1952), and the value of the nitrogen-oxygen dose ratio is approximately 2.5. This value can be compared with the nitrogen-oxygen dose ratio for chromatid exchanges with neutrons of 2.3 and with alpha particles of approximately 1 (table 3). Despite the variations which exist in the nitrogen-oxygen dose ratios for the different aberration types within both the X-ray and neutron experiments, it is clear that in all comparisons the oxygen effect is less pronounced with neutrons than it is with X-rays. The possible significance of these variations is discussed elsewhere (RILEY, GILES and BEATTY 1952).

On the basis of the data presented in table 3, it appears that the oxygen effect with fast neutrons is intermediate in magnitude between that found with X-rays and alpha particles for all types of aberrations for which data are available. Further, it is evident that there is an inverse relationship between the specific ionization (ionization density) produced by a given type of radiation and the magnitude of the oxygen effect on chromosome aberration production.

The original results with Vicia comparing X-rays and alpha particles were

interpreted as indicating that hydrogen peroxide is responsible for chromosome aberration production, since the yield of this substance in water irradiated with alpha particles is independent of the oxygen concentration, but increases markedly with increasing oxygen concentration in water irradiated with X-rays. Other parallelisms between hydrogen peroxide production and chromosome aberration production with X-rays may also be cited (cf. THODAY and READ 1949; GILES 1952). The present results with fast neutrons may also be interpreted on the peroxide hypothesis if the effect of oxygen on hydrogen peroxide production by fast neutrons is intermediate between that with X-rays and alpha particles. There appear to be few quantitative experimental data on this point, but the results of ALLEN (1948) indicate that hydrogen peroxide does occur in oxygen-free water irradiated with neutrons.

It appears more probable, however, that hydrogen peroxide alone is not responsible for the entire radiation effect, particularly following X-irradiation, either in the presence or in the absence of oxygen. With this radiation it seems very likely that the hydroperoxyl radical (HO_2), which is produced by the reaction of dissolved oxygen with H atoms, plays a major role when oxygen is present, even though hydrogen peroxide molecules resulting from the subsequent reactions of these radicals with additional H atoms may also produce some of the biological effect. Thus, it may well be that reactions produced by OH and HO_2 radicals (and possibly by H atoms), rather than by hydrogen peroxide, are principally effective in producing chromosome aberrations and that a certain average concentration of such radicals may be required for a chromosome break to result. The existence of an inverse correlation between ionization density and the effect of oxygen could then be interpreted as resulting from competitive interactions, involving the primary radicals or atoms (OH and H) produced in irradiated water, and molecular oxygen. The radiochemical evidence (LEA 1947; GRAY 1949) indicates that the distribution of these primary products (OH and H) will be quite different for various radiations and will resemble in general the pattern of primary ionization distribution. Thus, following the passage of an alpha particle through water there will arise a high concentration of H atoms and OH radicals close to the axis of the particle path. The distribution of H atoms will presumably be somewhat more peripheral, but will still be relatively concentrated. Conversely, following X-irradiation, both OH radicals and H atoms will be more widely spaced (except near the end of the secondary electron path). These distributions following fast neutron irradiation would be intermediate. Under these circumstances, the high concentration of OH radicals produced by alpha radiation would favor efficient break production. Further, the relative proximity of H atoms would favor their recombination to form molecular hydrogen. Thus, even with oxygen present, there would be little opportunity for HO_2 radicals to be formed with alpha radiation, and consequently little or no effect of oxygen on aberration yield. Following X-irradiation a different situation would exist. The relative concentration of OH radicals would be less and the efficiency of break production consequently reduced. However, the more widely

spaced distribution of H atoms would make their recombination less likely, and with oxygen present would favor HO_2 production and a consequent increase in aberration yield. With fast neutrons, an intermediate distribution of radicals should arise, depending on the energies of the recoil protons, and some oxygen effect might be expected. In summary, on this interpretation, the presence of oxygen during irradiation would have little effect when radiations having a high specific ionization were used, since under these circumstances the reaction $H + H \rightarrow H_2$ would be favored over $H + O_2 \rightarrow HO_2$. With radiations having a low specific ionization, the opposite situation would obtain and a pronounced effect of oxygen would result.

It should be pointed out, however, that the possibility also exists that hydrogen peroxide molecules may be involved in alpha particle effects, even if they prove to be relatively unimportant in X-ray effects, since the close proximity of OH radicals along the paths of these particles should favor their rapid reaction to form hydrogen peroxide. At the present time it does not appear possible to decide whether OH radicals or hydrogen peroxide molecules are primarily responsible for the alpha-particle effects. Further, there is the additional possibility that active products of secondary reactions, such as organic peroxides which are known to be mutagenic (DICKEY *et al.* 1949), are involved. This seems rather unlikely, however, in view of the apparent localization of the effects to the immediate vicinity of particle paths, a point which will be discussed later.

Previous experiments (GILES and BEATTY 1950) on the relationship between the percentage of oxygen present during X-irradiation and aberration frequency demonstrated that there is still an appreciable aberration yield even when oxygen is removed as completely as possible from the cells before irradiation. Such residual aberrations could arise either from the indirect effects of OH radicals produced in oxygen-free water, as has been assumed in the preceding discussion, or from the direct effect of the radiation on the chromosomes. In an experimental attempt to distinguish between these alternatives, irradiations were performed in hydrogen under pressure in order to facilitate the removal of OH radicals by promoting the back reaction: $H_2 + OH \rightarrow H_2O + H$. This treatment did not reduce the aberration frequency significantly (GILES and BEATTY 1950). The failure to find a hydrogen effect may be interpreted as indicating that OH radicals are not involved in aberration production. However, since it has not been experimentally verified that the postulated back reaction actually can occur in the biological system under test, the negative evidence from this experiment is not conclusive. It still seems likely in view of the high water content of this system, that OH radicals are responsible (at least in part) for break production in the absence of oxygen.

It will be recalled that the relationship between interchange frequency (both chromosome and chromatid) and fast neutron dosage is linear whether the irradiation takes place in the presence or in the absence of oxygen. For X-rays, however, this relationship is nonlinear in both instances. These data provide further evidence for a relative localization of radiation effects, whether such

effects are produced directly or indirectly. Even though indirect action by way of radical formation may be involved, both breaks resulting in an interchange must be produced in most instances by radicals arising along a single particle path with neutrons, but along two separate particle paths with X-rays.

SUMMARY

An experiment has been performed to determine the effect of oxygen on the production by fast neutrons (derived from uranium fission in the Oak Ridge nuclear reactor) of chromosomal aberrations in Tradescantia microspores. Inflorescences were exposed in a special airtight lucite chamber in atmospheres of either pure oxygen, pure nitrogen, or pure helium. Studies were made of both chromatid aberrations (at 24 hours following treatment) and chromosome aberrations (at four and five days after treatment).

The results of this experiment indicate that, in the absence of oxygen, the frequencies of all types of aberrations (in both chromosome and chromatid categories) are reduced when equal dosages of fast neutrons are compared. However, a quantitative comparison of these results with previous observations on the oxygen effect with X-rays and with alpha particles in Tradescantia and Vicia indicates that this effect with neutrons is intermediate, being greater than that found with alpha particles and less than that found with X-rays. There is thus an inverse relationship between the specific ionization (ionization density) produced by a given type of radiation and the magnitude of the oxygen effect on chromosome aberration production. This relationship is interpreted in terms of differential spatial distributions, and consequent interactions, of the primary radiation products (OH radicals and H atoms), in the presence of oxygen, following the exposure of cells to various types of radiations.

The relationship between fast neutron dosage and aberration frequency is linear for all types of aberrations (including both chromatid and chromosome interchanges) in both the presence and absence of oxygen. This finding provides further evidence for a relative localization of radiation effects to the immediate vicinity of particle tracks within a cell whether such effects are considered to be produced indirectly or directly.

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