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Health and Biology
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Norman H. Giles, Jr.

DATE ISSUED
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RECENT EVIDENCE ON THE MECHANISM OF CHROMOSOME ABERRATION PRODUCTION BY IONIZING RADIATIONS

Norman H. Giles, Jr.
Biology Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee

An understanding of the mechanism by which ionizing radiations produce chromosome aberrations is one of the fundamental problems in radiation genetics. The present discussion will deal with recent evidence on this subject, obtained for the most part from experiments with plant chromosomes, especially those of the spiderwort, Tradescantia. At the outset it appears desirable to review briefly the interpretation of aberration production in Tradescantia as proposed originally by Sax (1938, 1940) and developed in quantitative form primarily by Lea (1946). Following this, more recent results, stemming principally from the observations by Thoday and Read (1947, 1949) on the effect of oxygen on aberration frequency, will be presented. Finally the implications of the oxygen effect for an interpretation of the biochemical mechanism of radiation-induced chromosomal changes will be considered, in particular, the extent to which this effect requires a revision of previous views as to the mechanism involved.

The Production of Chromosomal Aberrations in Tradescantia by Ionizing Radiations.—The general experimental technique for observing the effects of ionizing radiations on Tradescantia chromosomes as developed by Sax (1938) has been described in detail previously (Sax, 1940; Catcheside, Lea, and Thoday, 1946a and b; Lea, 1946) and will be considered here only briefly. The typical procedure is to expose entire inflorescences, consisting of several buds containing microspores in various stages of development, to penetrating radiations such as X rays or fast neutrons. Cytological examinations are then made at appropriate intervals after treatment to detect aberrations at the first postmeiotic mitosis in the microspore. Two general categories of aberration types are noted —

(a) chromatid types, resulting from irradiation of chromosomes which are effectively double in prophase, and (b) chromosome types resulting from irradiation of chromosomes which are effectively single in resting stage. In both instances, radiation produces breaks in one or more of the six chromosomes in the nucleus of a microspore and the resulting broken ends may either remain as such, undergo restitution, or rejoin with other broken ends to produce aberrant configurations which are cytologically detectable. The principal configuration types with which we shall be concerned in the experimental results to be discussed in this paper may be designated as chromosome interchanges. These rearrangements, observed in cells examined 4 to 5 days following irradiation, are either dicentric or ring chromosomes and result from breaks in two separate, undivided chromosomes, or chromosome arms, followed by the reunion of broken ends to give one chromosome with two centromeres, or one continuous ring chromosome, plus an accompanying acentric fragment in each case.

The essential features of the hypothesis developed by Sax, Lea, and others to explain the production of chromosome aberrations in Tradescantia may be outlined as follows: electromagnetic or particulate radiations produce their effects as a consequence of the formation of ion pairs within a chromosome during the passage through the chromosome of either primary or secondary charged particles. Chemical changes resulting from this direct ionization of the molecules composing the chromosome lead to the production of chromosome breaks. The resulting broken ends may remain as such yielding terminal deletions, or undergo restitution giving rise to apparently normal chromosomes again, or rejoin with other broken ends producing cytologically visible aberrations. The restitution and reunion processes are competitive and both space and time factors are involved. Several ionizations (between fifteen and twenty) must occur within the chromosome to produce a break and the factor of major consequence in distinguishing the quantitative effects of

various radiations is the difference in ionization distribution along particle tracks. For certain radiations (e.g., gamma rays and X rays) ionization distribution along the tracks (of secondary electrons) is such that the probability of breakage of a chromosome by a traversing particle is considerably less than 1 for most of the length of the track except near the end. As a consequence, such radiations are relatively inefficient in producing breaks on the basis of total ionization produced per track. Furthermore, breaks in two separate chromosomes (or chromosome arms) are almost always produced by two separate particle tracks, with the result that the frequency of interchange aberrations (e.g., dicentrics and rings) increases as the square of the dose (a two-hit curve) when the time of irradiation is kept constant. Because such aberrations are two-hit phenomena, there is also an intensity effect — the yield of interchanges decreasing when a constant dosage of radiation is administered over periods of increasing duration. The average time during which a break may remain "open" before restitution or reunion occurs (i.e., its half life) is at least 4 minutes.

For other radiations (e.g., recoil protons from fast neutrons), ionization distribuion along tracks is such that the probability of breakage of a chromosome by a traversing particle is close to 1, and as a consequence the efficiency of such radiations in producing chromosomal changes is very high compared to gamma or X rays. In addition, both of the breaks in the two separate chromosomes (or chromosome arms) taking part in an interchange are usually produced by a single particle track, resulting in a linear relationship between interchange frequency and dose (a one-hit curve) and the absence of an intensity effect.

In addition to aberrations resulting from breaks in two separate chromosomes, certain types are produced by breaks in a single chromosome arm (either divided or single). The majority of these aberrations are one-hit types resulting from the passage of a single ionizing particle, and exhibit no intensity effect with any type of radiation.

The interpretation of chromosome aberration production as just outlined has been quite generally successful in accounting for most of the quantitative results of radiation experiments with Tradescantia. Recently, however, experimental data of two sorts have been obtained which indicate that this hypothesis in its simplest form is not entirely adequate. The first evidence was that obtained by Kotval and Gray (1947) in their studies with alpha particles. On the basis of comparative ionization distribution and particle numbers, the hypothesis predicts that a given amount of ionization produced by alpha particles should be considerably less efficient in producing chromosome breaks than an equal ionization dose produced by fast neutrons, whereas the experimental results indicate that for equal ionization doses alpha particles are somewhat more efficient. It was concluded that a proportion of the breaks produced by alpha particles arises from ionization produced in the immediate vicinity of, but not within, a chromosome, thus suggesting the involvement of an indirect as well as a direct mechanism. The second, and even more striking, evidence was that obtained by Thoday and Read (1947), who noted a pronounced effect of oxygen on the frequency of X-ray-induced aberrations in the root-tip mitoses of the broad bean, Vicia faba. Their experiments indicated that the absence of oxygen during irradiation resulted in a marked decrease in aberration frequency. Similar results were obtained by Hayden and Smith (1949) in experiments with barley seeds. Experiments on the effect of oxygen have also been perfomed with Tradescantia and the results of some of these will now be discussed.

The Effect of Oxygen on X-Ray-Induced Chromosomal Rearrangements in Tradescantia.--The experimental methods used to expose Tradescantia inflorescences to X radiation while in atmospheres containing various percentages of oxygen have been described in some detail elsewhere (Giles and Riley, 1949, 1950) and will be outlined only briefly here. Inflorescences were placed in an appropriate holder

inside an airtight lucite exposure chamber. This chamber was placed inside the X-ray machine and connected through ports by pressure tubing and appropriate valves to a vacuum pump, a gas pressure cylinder, and a mercury manometer. Air in the chamber could be evacuated and replaced by the appropriate gas or gas mixture from the cylinder. The chamber could also be maintained under vacuum, or under pressures up to three atmospheres above normal atmospheric. Rapid introduction or removal of gas could be effected with the apparatus. All these manipulations could be carried out before, during, or after irradiation, depending on the experimental conditions desired. The X-ray intensity for each exposure was determined by means of a Victoreen thimble ionization chamber which could be inserted into the box to the same position as that normally occupied by the inflorescences.

A series of dosage curves was obtained for inflorescences exposed in air, in oxygen, and in nitrogen. The yield of both interchanges (dicentrics and rings) and interstitial deletions was markedly reduced when nitrogen replaced air in the chamber, and increased somewhat when oxygen replaced air. Additional comparative exposures were made in other gases, such as helium and argon, and also under vacuum. In all instances, reduced aberration frequencies similar to those obtained in nitrogen resulted, indicating that the absence of oxygen was responsible for the decrease in radiosensitivity (Giles and Riley, 1949). No chromosomal effects were noted in control experiments in which similar exposures to nitrogen and oxygen were made without irradiation. It was clear from these results that the presence of oxygen resulted in a marked increase in aberration frequency.

The next problem was to determine the reason for this effect of oxygen. If it is assumed that all breakage is the result of direct-hit effects, the same number of breaks should be produced in the presence or absence of oxygen by a given X-ray dose. Thus the increased aberration frequency obtained in oxygen might

result from an effect of oxygen itself on the recovery process, such that when oxygen is present new reunions of broken ends are favored as opposed to restitution. It seemed possible, for example, that such an effect could result from the stimulation of chromosome movement by oxygen. Another and perhaps more likely possibility appeared to be that the effect of oxygen is an indirect one, such that in the presence of dissolved oxygen, X rays produce in cells a certain substance or substances which increase the yield of aberrations. In this event, such an intermediate substance could produce an effect by way of either the recovery or the breakage mechanism. In the former instance, the X-ray breakage of chromosomes would still be considered as a direct effect; in the latter, however, the breakage would have to be considered as an indirect effect, at least in part.

It appeared feasible to attack some of these problems experimentally in Tradescantia, since in this organism the recovery process extends over a considerable period of time (the average time between production of a break and restitution or reunion being at least 4 minutes). Thus it is possible to separate to a considerable degree the two processes of breakage and recovery and to test the effect of oxygen on each. To do this, inflorescences were exposed to a single dose of 300 r of X rays in 1 minute, either in the presence of pure oxygen or in the absence of oxygen (in vacuo). Immediately after the irradiation oxygen was either removed (by evacuation) or introduced (to a positive pressure of 1500 mm of Hg). The exchange of gases, as recorded by the manometer, could be effected quite rapidly, and in this fashion it was possible to have the breakage process occurring in oxygen and recovery largely in its absence, or the reverse. In addition, other experiments were performed in which oxygen was either introduced or removed during part of the X-ray exposure. The results of such comparative exposure are reported in the paper of Giles and Riley (1950). Recently certain other experiments of a similar type have been performed and these results will be presented here. In the original experiment in which a single exposure of 1 minute

to 300 r was made in vacuum followed by the immediate introduction of oxygen, no effect on the recovery process was noted. It was decided to increase the possibility of detecting such an effect by fractionating the dose so that a relatively larger portion of the recovery period would take place in oxygen. The following procedure was used: a set of inflorescences was exposed in vacuum for 20 seconds at 300 r/min.; following the irradiation, oxygen was immediately introduced into the chamber to a positive pressure of 1500 mm of Hg and allowed to remain for 8 minutes; the chamber was then reevacuated and another 20 seconds exposure made, followed by the reintroduction of oxygen as above; this procedure was repeated five times to give a total dose of 500 r. Two control series were run, in one of which the procedure just described was followed except that helium rather than oxygen was introduced. In the other control a total dose of 500 r was administered in 100 seconds of continuous exposure in vacuum; helium was then introduced and remained in the chamber for 8 minutes after the X-ray exposure. The data from this experiment are presented in Table 1. It is evident that there is no increase in aberration frequency in series A, in which oxygen was present during recovery, as compared with B, in which helium was present. The somewhat higher values for C are to be expected since this was a continuous exposure with no intervening recovery periods.

A second experiment has been performed (in cooperation with A. V. Beatty) to retest the observation (Giles and Riley, 1950) that the addition of oxygen during irradiation results in an immediate increase in aberration frequency. These data are presented in Table 2. In this instance, essentially the same experimental conditions were utilized as previously, except that a second exposure (Series C) was made in which oxygen was present during only the last 15 seconds of the total X-ray exposure of 1 minute. The results of this experiment are in agreement with the earlier one in indicating that the introduction of oxygen during irradiation

Table 1

Test of the effect of oxygen on the recovery mechanism, using fractionated doses. All series received a total dose of 500 r at 300 r/min. In Series A this dose was delivered in five equal fractions, with irradiation occurring in vacuum, and each intervening recovery period of 8 minutes occurring in oxygen at a positive pressure of 1500 mm of Hg. Series B was similar, except that the recovery periods occurred in helium. Series C received one continuous exposure in vacuum, with recovery occurring in helium.

Series No.	Conditions	No. Cells Examined	Interchanges Per Cell	Interstitial Deletions Per Cell
A	Fractioned dose; irradiation in vacuum; recovery in oxygen.	323	0.23 ± 0.027	0.20 ± 0.025
B	Fractioned dose; irradiation in vacuum; recovery in helium.	450	0.28 ± 0.025	0.31 ± 0.026
C	Continuous exposure; irradiation in vacuum; recovery in helium	582	0.31 ± 0.023	0.35 ± 0.024

Table 2

Experiments on the introduction of oxygen during X irradiation of Tradescantia inflorescences. All exposures of 300 r at 300 r/min.

Series No.	Pretreatment Conditions	Exposure Conditions	Posttreatment Conditions	No. Cells	Interchanges Per Cell	Interstitial Deletions Per Cell
A	Buds in vacuum	Vacuum	Vacuum - 10 min.	850	0.11 ± 0.01	0.08 ± 0.01
B	Buds in vacuum	1st 30 sec; vacuum 2nd 30 sec; oxygen introduced (within 3 sec.) to 1500 mm of Hg	Evacuation (within 25 sec.); vacuum - 10 min.	900	0.32 ± 0.02	0.36 ± 0.02
C	Buds in vacuum	1st 45 sec; vacuum last 15 sec; oxygen introduced (within 3 sec.) to 1500 mm of Hg	Evacuation (within 25 sec.); vacuum - 10 min.	600	0.22 ± 0.02	0.26 ± 0.02
D	Buds in oxygen	Oxygen at 1500 mm of Hg	Evacuation (within 25 sec.); vacuum - 10 min.	900	0.61 ± 0.03	0.67 ± 0.03

results in an immediate increase in aberration frequency.

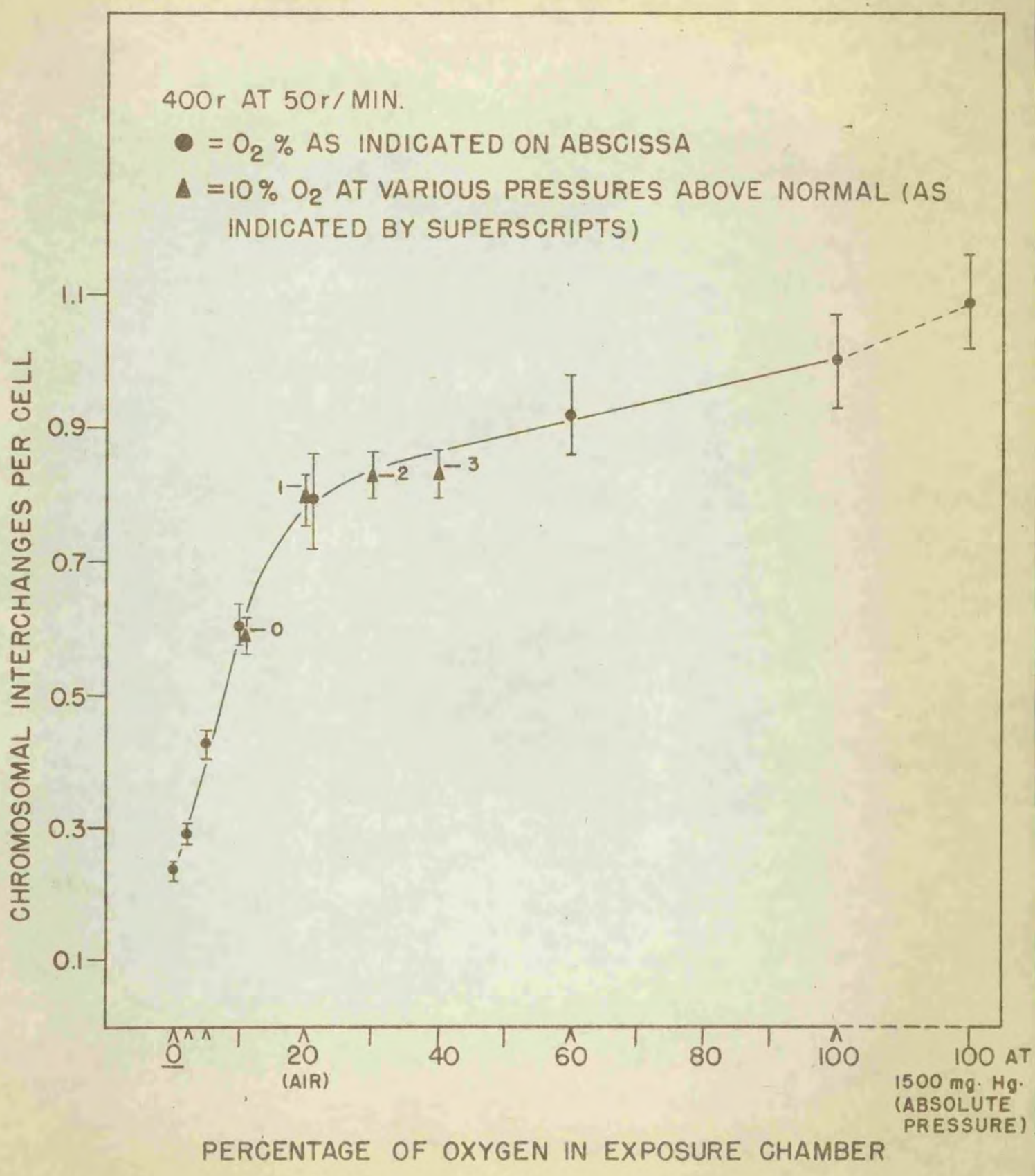
It is clear from these comparisons (and those reported earlier) that the addition or removal of oxygen immediately after irradiation does not modify the aberration frequency, thus indicating that oxygen itself has no effect on the recovery process. There seems to be little question that, under the experimental conditions utilized, oxygen does diffuse very rapidly into the cells and is present during the recovery process. This is shown by the fact that the introduction of oxygen during irradiation causes a pronounced increase in aberration frequency. This latter result is also important in providing additional evidence that oxygen to be effective must be present during the actual irradiation and that there is little or no latent period between the introduction of the gas and its effect in terms of increased aberration production. Other experiments have also demonstrated that a preexposure of buds in pure oxygen before they are irradiated in helium has no effect.

From all these observations, it seems clear that the effect of oxygen itself is an indirect one presumably arising from the production by X rays when oxygen is present, of some substance which increases the frequency of aberrations. As indicated previously, this effect of such a substance might be by way of either the breakage or the recovery mechanism. The most likely hypothesis seems to be that such a substance would cause an increased production of chromosome breaks. However, the alternative possibility that the recovery process is modified, cannot be immediately excluded on the basis of the data just presented. This problem must be attacked by other methods. Experiments designed to determine whether the recovery time is different for breaks produced in the presence and absence of oxygen are under way (Giles and Riley, unpublished). The results to date are compatible with the view that the recovery mechanism is essentially the same under these two conditions. The previously reported differences in the slopes

of dosage curves obtained at a constant intensity of 45 r/min. in air, in oxygen, and in nitrogen, which at first appeared to be due to an effect on restitution time, can be explained on the basis of an intensity effect resulting from a lesser production of breaks per unit time in nitrogen. It has been shown by Sax (1941) that the exponents of dosage curves from chromosome interchanges decrease with decreasing radiation intensity. Evidence has also been obtained (Giles and Beatty, unpublished) that the effect of temperature, which has been previously interpreted as influencing the recovery process (Sax, 1940), is actually, at least to a considerable extent, an indirect effect on oxygen availability. Experiments with Drosophila (Baker and Sgourakis, 1950) have demonstrated that oxygen has a marked effect in increasing the frequency of other types of X-ray-induced genetic changes, sex-linked lethal mutations, where there is no evidence that a recovery process is involved. All these results suggest very strongly that the oxygen effect is on the breakage and not on the recovery mechanism in Tradescantia.

In order to provide additional evidence on the mechanism of the oxygen effect, it appeared desirable to determine the relationship between the amount of oxygen present during irradiation at a constant dosage and aberration frequency. Consequently, a series of exposures has been made of inflorescences in atmospheres containing different percentages of oxygen (mixed with helium) at a single X-ray dose of 400 r. Some data on this point have already been published (Giles and Riley, 1950). Another experiment (Giles and Beatty, unpublished) has been carried out to determine this relationship more precisely. In this instance special attempts were made to free the helium used of any residual oxygen. The percentages of oxygen (2, 5 and 10 per cent) in the other gas mixtures used were accurate to within ± 0.2 per cent and a larger number of cells was scored to increase the statistical reliability of the determinations. A graphical summary of the results is presented in Figure 1, together with the averages of points obtained at higher percentages

FIG. 1


400r AT 50r/MIN.
● = O_2 % AS INDICATED ON ABSCISSA
▲ =10% O_2 AT VARIOUS PRESSURES ABOVE NORMAL (AS INDICATED BY SUPERSCRIPTS)
CHROMOSOMAL INTERCHANGES PER CELL
1.1
0.9
0.7
0.5
0.3
0.1
0
1
2
3
0
20 (AIR)
40
60
80
100
100 AT 1500 mg. Hg. (ABSOLUTE PRESSURE)
PERCENTAGE OF OXYGEN IN EXPOSURE CHAMBER

of oxygen in previous experiments. It is clear that there is still a substantial yield of aberrations even in the complete (or nearly complete) absence of oxygen. When oxygen is present in irradiated cells, there is a rapid rise in aberration frequency above this base level. This increase is linear between 0 and 10 per cent oxygen, after which the rise is apparently more gradual.

Certain additional experiments (Giles and Beatty, unpublished) provide further evidence that the amount of dissolved oxygen present in the cells is an important factor in determing aberration frequency. In these experiments, a constant percentage of oxygen was used in the exposure chamber, but irradiations were carried out with the inflorescences under pressures up to 3 atmospheres above normal. The data obtained for exposures at 0, 1, 2, and 3 atmospheres above normal pressure (approximately 740 mm of Hg) in 10 per cent oxygen (plus 90 per cent helium) have been indicated in Figure 1, on the assumption that the amount of effective dissolved oxygen in the cells is directly proportional to the pressure. Control experiments in which comparable exposures were made in helium under pressure indicated that pressure alone did not change the aberration frequency. As can be seen from the graph (Fig. 1) there is good agreement with previous exposures in different percentages of oxygen at normal atmospheric pressure. As mentioned previously, preliminary experiments have also been completed which strongly suggest that the effect of temperature on aberration frequency is, at least in large part, actually an oxygen effect (Giles and Beatty, unpublished). Similar evidence concerning the temperature effect on sex-linked lethals in *Drosophila* has already been obtained by Baker and Sgourakis (1950).

The Biochemical Mechanism of the Oxygen Effect.—It is clear from the results which have just been presented that oxygen has a marked effect in increasing the radiosensitivity of *Tradescantia* chromosomes, as measured by the frequency of X-ray-induced interchanges and deletions. We shall assume, as most of the evidence

seems to indicate, that this effect arises only if oxygen is present in cells during the actual period of irradiation, and results from the production by X rays of more chromosome breaks under these circumstances. The simplest explanation for this situation would appear to be that in the presence of oxygen, irradiation results in the production within the nucleus of some substance (or substances) which causes an increase in chromosome breakage and that the amount of this substance produced is positively correlated with the amount of oxygen present. It thus becomes of interest to determine, if possible, what this substance is. Since these cells are composed largely of water, it seems very probable that the substance is a product of irradiated water, more particularly, a product characteristically formed when oxygen is present in irradiated water. It has already been suggested by Thoday and Read (1947, 1949), that this product may be hydrogen peroxide. Additional evidence is now available which supports this conclusion. The results of four entirely unrelated types of experiments furnish evidence favoring or compatible with the H_2O_2 hypothesis. Bonet-Maury and Lefort (1948) have recently investigated the production of H_2O_2 in water irradiated with X rays and with alpha particles under various conditions, including the effect of oxygen concentration and of temperature. In addition, data are available on the effect of pH on peroxide yield (Loiseleur, 1942). At least four striking parallels exist between H_2O_2 production and chromosome aberration production under varying conditions of irradiation. (1) It is found that with X rays H_2O_2 is not produced in oxygen-free water (or is produced in very small amounts, as shown by Allen, 1948), but that when oxygen is present the amount of H_2O_2 produced depends markedly on the concentration of oxygen. The type of curve obtained for the increased yield of H_2O_2 with increasing oxygen concentration at a constant X-ray dose is generally similar to that obtained in the present studies for the relation between aberration frequency and the percentage of oxygen present during X radiation.

(2) In oxygen-saturated water, H_2O_2 production by X rays decreases regularly with decreasing temperature, a definite discontinuity marking the passage from water to ice. Below -116° C no H_2O_2 can be detected. Fabergé (1950) has shown that when Tradescantia pollen grains are X-rayed at various temperatures, the general character of the sensitivity curve (as measured by the number of chromosome breaks observed in pollen tube divisions) resembles that for H_2O_2 production. There is a dip in the region of 0° C and a gradual decline thereafter. However, although H_2O_2 production stops at -116° C, chromosome breaks are still produced at -192° C, their frequency being almost one-fifth that at +25° C. (3) The pH of the solution exerts an effect on the yield of H_2O_2 with X rays, an alkaline pH favoring a lower H_2O_2 concentration. The experiments of Marshak (1938) have shown that the frequency of chromosome aberrations observed at anaphase in root tips of Vicia faba and Allium cepa is markedly reduced when X radiation is carried out in the presence of penetrating bases, such as ammonium hydroxide. (4) Undoubtedly the most cogent evidence obtained to date in favor of the H_2O_2 hypothesis is that derived from a comparison of X-ray and alpha-particle effects in the presence and absence of oxygen. With alpha particles, H_2O_2 production occurs even in oxygen-free water and the addition of oxygen does not increase the yield. Thoday and Read (1949) have shown that, for aberrations induced in the root tips of Vicia faba, the removal of oxygen results in little if any decrease in aberration frequency when the cells are irradiated with alpha particles as compared with a very marked decrease when X rays are used. These observations have been confirmed in preliminary results obtained by Conger (unpublished) in experiments on the irradiation of mature pollen grains of Tradescantia with alpha particles.

Although these comparisons suggest very strongly that H_2O_2 may be the product involved in the oxygen effect, they do not establish this point unequivocally. The possibility exists that other products of the radiodecomposition of water may

be concerned. The recent observations of Krenz and Dewhurst (1949) on the effect of dissolved oxygen on the oxidation of ferrous sulfate in aqueous solution by gamma rays can apparently best be explained by a mechanism involving the HO_2 radical. The marked similarity between the magnitude of the decreased oxidation of ferrous sulfate in the absence of oxygen (to about one-fourth) and the decrease in aberration frequency obtained in the early experiments with Tradescantia is noteworthy. However, the absolute magnitude of the decrease in aberration frequency occurring in the absence of oxygen apparently depends on the dose and the intensity in a particular experiment. Further, the experimental results with alpha particles in the presence and absence of oxygen appear to make it unlikely that HO_2 is the intermediate involved. These results are in agreement with the view, on radiochemical grounds, that H_2O_2 is formed directly in large amounts by alpha particles because of the very close proximity of the OH radicals produced in the center of the particle track (Gray, 1949). The failure of O_2 to increase the yield of H_2O_2 apparently indicates that the usual reaction for peroxide formation by way of the intermediate HO_2 radical is relatively unimportant (Bonet-Maury and Lefort, 1948). It thus appears that H_2O_2, rather than HO_2 may be responsible for chromosome breakage. There are, however, additional mechanisms by which HO_2 may be produced from peroxide, and the possibility cannot be entirely eliminated that this radical may also be to some extent an effective agent in chromosome breakage.

Additional evidence on the presumptive role of H_2O_2 should be obtained from experiments with enzyme inhibitors such as cyanide which should block the action of catalase and permit H_2O_2 accumulation. A suggestive slight mutagenic effect of cyanide alone, which has been interpreted on this basis, has already been reported by Wagner et al. (1950) in Neurospora. The experiments of Mottram (1935) in which cyanide increases the sensitivity of roots of Vicia faba to X rays, as

judged by inhibition of growth, also lend support to this possibility. In the Neurospora experiments a direct mutagenic effect of H_2O_2 was also noted. In experiments utilizing enzyme poisons to elucidate the mechanisms of the oxygen effect, however, the specificity of the poison would appear to be exceedingly important, otherwise an unequivocal interpretation of the results is not possible. Effects due to cyanide might result from an inhibition of the cytochrome system thus preventing the utilization of oxygen by the respiratory enzyme systems of the cell. If such a utilization of oxygen is necessary to bring about its effect on radiosensitivity, then it might be expected that cyanide would decrease, rather than increase radiosensitivity. There is, in fact, one report that cyanide does exert a protective action against the killing of mice by X rays (Bacq et al., 1950), but these results are not confirmed in similar experiments with rats (Dowdy et al., 1950) in which a clear effect of anoxic anoxia was demonstrated. With respect to the production of chromosomal aberrations, it appears probable that the oxygen effect is produced by oxygen dissolved in the aqueous medium of a cell, but additional experimental evidence on this point is being sought.

There is also the possibility that even though H_2O_2 may be one of the essentially primary radiation products associated with the oxygen effect, it still may not be the actual mutagen directly responsible for chromosome breakage. It might be only an intermediate in the formation of other substances such as organic peroxides, some of which have recently been shown to have marked mutagenic effects in Neurospora (Dickey et al., 1949). It appears likely that the effect of organic peroxides may result from free radical formation, and indeed, it is possible that most, if not all, chemical mutagenic effects may be explicable on this basis and thus turn out to be fundamentally related to radiation-induced mutations (Auerbach, 1949; Dickey et al., 1949; Jensen, et al., 1949).

The Relation of the Oxygen Effect to Previous Views on the Mechanism of Chromosome Breakage in *Tradescantia* by Ionizing Radiations.—The preceeding discussion has indicated the remarkable effect of oxygen in increasing the radiosensitivity of *Tradescantia* chromosomes. This effect can be most easily interpreted as resulting from an increased production of chromosome breaks by X radiation, the amount of increase being positively correlated with the amount of oxygen present in cells. On the basis of such results, it would appear that the previous hypothesis utilized to explain the production of aberrations in *Tradescantia* must be modified. On this hypothesis, as outlined earlier, chromosome breakage has been considered to result from the direct action of the radiation in ionizing the molecules actually composing a chromosome, as a consequence of the passage through the chromosome of an ionizing particle (Lea and Catcheside, 1942). It now appears most likely that an indirect mechanism is involved, in which irradiation of the oxygen-containing aqueous medium in the cell leads to the production of some substance which in turn produces chromosome breaks.

It should be recalled, however, that a substantial aberration frequency is still produced by irradiation in the absence of oxygen (at least in so far as oxygen can be removed from these cells). The question thus arises whether there are two mechanisms for chromosome break production, one involving direct ionization of the chromosome molecules and the other an indirect effect from the irradiated aqueous medium, and further whether the relative importance of the two mechanisms may be judged by the degree of the oxygen effect. That such is the situation is by no means clear. It seems possible, in fact, that at least some of the aberrations induced in the absence of oxygen may also be the result of an indirect effect, being produced by substances other than H_2O_2 or HO_2, such as OH radicals, resulting from the radiodecomposition of essentially oxygen-free water.

Attempts have been made to test this point by experiments (Giles and Beatty,

unpublished) designed to minimize the effectiveness of the OH radical by promoting, during irradiation, the back reaction to form H_2O (Allen, 1948). To do this, inflorescences were exposed to 400 r of X rays in atmospheres of hydrogen, both at normal pressure and at three atmospheres above normal. Interchange frequencies were essentially the same for the two exposures and although both values were somewhat lower than those obtained in comparable exposures in helium or nitrogen, the difference is not significant. If it is valid to assume that hydrogen would in fact react to remove OH radicals formed during irradiation, the failure to detect a reduced aberration yield in these experiments supports the view that chromosome breakage produced by X rays in the absence of oxygen may all result from direct ionization of the chromosome molecules. It should be pointed out, however, that this conclusion is based on the assumption that reactions leading to H_2O_2 production or suppression in cells from which oxygen has been removed as completely as possible are similar to those occurring in oxygen-free pure water. There is as yet little experimental evidence on this point and it is quite possible that the complexity of the cellular environment may cause very different reactions to occur.

Unfortunately it does not appear to be experimentally feasible to assess the relative importance of the indirect and direct effects on chromosomes by the method normally employed for enzymes and viruses — that of determining the effect of a constant radiation dose when the solute concentration is varied over a considerable range. The nearest approach to this situation would appear to be sensitivity tests of cells that vary in water content. This is not possible with developing microspores of the type used in these experiments. It might be feasible with mature pollen grains, however. There are in fact many data which indicate that dry seeds of plants show increased radioresistance, both with respect to killing and to induced genetic changes, genic as well as chromosomal, as compared with

hydrated seeds (Gustafsson, 1947). However, it is not always possible from such comparisons to conclude that the sensitivity changes are directly correlated with water content, and do not result from changes in the mitotic state of the nucleus, which are known to affect radiosensitivity.

Regardless of the degree of importance of the direct effect, it appears probable that in Tradescantia, at least, the magnitude of the indirect effect is considerably greater. However, it is still possible to interpret the results in terms of target theory, as indicated by Thoday (1950). The essential requirement is that the action of ionizing particles, whether direct or indirect, be relatively localized. If the effect is principally indirect, it appears that a substance (such as H_2O_2) must be produced along the track of an ionizing particle and must have a relatively limited effective diffusibility (or short half life). In fact it seems necessary that its effective distribution within the nucleus must correspond in pattern rather closely to that of ionization distribution along particle tracks. Such a localized distribution would appear to be essential, as has been pointed out by Zirkle (1949), in order to explain the striking quantitative differences among various radiations, as, for example, the shapes of the dosage curves for interchanges induced by X rays and alpha particles.

Summary: Recent experiments have demonstrated that oxygen has a marked effect in increasing the sensitivity of chromsomes in Tradescantia and other plants to X rays as measured by the frequency of cytologically detected aberrations. It has been shown that this is not an effect of oxygen itself on the behavior of broken ends of chromosomes. The effect apparently arises from the production by X rays, as a result of the radiodecomposition of water in cells containing oxygen, of some substance which causes an increase in aberration frequency. Several independent lines of evidence indicate that this substance may be H_2O_2. It appears likely that the increased frequency of aberrations arises from an increased production of

chromosome breaks when oxygen is present during irradiation, rather than from a modification of the recovery process. Thus a major fraction of the radiation effect on Tradescantia chromosomes is to be considered indirect rather than direct. In the absence of oxygen, however there is still an appreciable aberration frequency and some evidence indicates that this entire fraction may be the result of direct radiation action.

Since the previous hypothesis explaining chromosome breakage in Tradescantia assumed that all of the effect of the radiation resulted from the direct ionization of the molecules of the chromosome, the demonstration of an indirect effect necessitates a revision of this interpretation. However, it is still possible to interpret the results in terms of target theory. The essential requirement is that the action of ionizing particles, whether direct or indirect, be relatively localized. If the effect is principally indirect, it appears that a substance, such as H_2O_2, must be produced along the track of an ionizing particle and must have a relatively limited effective diffusibility (or short half life). In fact, it seems necessary that its effective distribution within the nucleus must correspond in pattern rather closely to that of ionization distribution along particle tracks.

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