THE EFFECT OF OXYGEN CONCENTRATION ON X-RAY-INDUCED CHROMOSOME BREAKAGE IN MAIZE*

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The effect of reduced oxygen tension in decreasing the radiosensitivity of biological material to x-rays has been demonstrated in many organisms. Experiments with onion,[1] Tradescantia,[2-4] Drosophila[5] and barley[6] have established that the production of x-ray-induced chromosomal aberrations is influenced by the amount of oxygen present in the cell at the time of irradiation. There are two ways by which the frequency of aberrations can be reduced, (1) by a reduction in the number of effective ionizations and (2) by an increase in the rejoining power of broken ends. The results of earlier investigations have been interpreted as favoring the first hypothesis. It was postulated that x-rays produce chromosome breaks in two ways, by ionization of the molecules of the chromosomes themselves and indirectly by ionization in the vicinity of the chromosome. In the latter case highly active intermediate products such as free radicals or peroxides are liberated which are capable of diffusing to the chromosome and causing breakage. Chromosomes irradiated in the presence of oxygen are thought to be broken by both mechanisms, while in reduced oxygen tension, the indirect effect is minimized.

The experiments described here were conducted to determine if oxygen affects the radiosensitivity of maize chromosomes. Evidence will be presented supporting the hypothesis that the effect of oxygen tension is on the recovery process rather than on the initial breakage mechanism.

Experimental Methods.—Genes *a* (aleurone color) and *sh* (shrunken endosperm) are situated on the long arm of chromosome 3. They are very closely linked giving 0.27% crossing over.[7] Pollen carrying both dominant genes was x-rayed at 1000 r and used to pollinate tester plants homozygous recessive for both genes but carrying all the other factors required for the expression of color in the aleurone, A_2, C and R. The exposures were made with a Collidge self-rectifying tube with a tungsten target, operated at 250 kv. and 15 ma. (3 mm. of Al, inherent filtration).

The pollen to be treated was collected from a number of plants, mixed thoroughly, and divided into two equal parts. One-half the pollen was irradiated in air and the other half in a 100% nitrogen atmosphere. The exposures were made in a lucite chamber. In the case of the nitrogen treatment, the chamber was made airtight and the pollen preflushed with the gas for 3 minutes. A steady flow of nitrogen was passed through the chamber during the $4^1/_2$ minutes of exposure.

Two classes of mutant kernels were observed, whole seed losses where the entire kernel showed only the recessive phenotype, and mosaics which were sectorial for areas of both dominant and recessive tissue. Approximately 3% of the kernels in the former class showed loss of only one of the two genes. All the kernels in the second class were mosaic for both genes.

Since closely linked genes were used in this study it is possible to distinguish the genetic effects due to chromosomal aberrations from those due to gene mutation. Losses of both *A* and *Sh* are due to chromosome breakage. Stadler and Roman[8] have shown that x-rays produce very few, if any, true gene mutations. Thus the chances that both genes *A* and *Sh* mutate simultaneously in the same pollen grain are practically nil. The single gene mutants are due to gene mutations or an internal deletion where one of the breaks occurred in the region between the two genes.

Results and Discussion.—A comparison of irradiation effects produced in nitrogen and in oxygen shows a decrease in the number of mutant kernels by a factor of 3.2 (table 1). This over-all protection value compares well with that obtained with Tradescantia and Drosophila. The data also indicate that there is a significant difference between the relative protection of

TABLE 1

FREQUENCY OF MUTANT KERNELS FOLLOWING IRRADIATION IN AIR AND NITROGEN

	NO. OF SEEDS	WHOLE LOSSES NO.	WHOLE LOSSES %	MOSAICS NO.	MOSAICS %	TOTAL, %
Air	6158	159	2.58	40	0.65	3.23
N_2	4505	43	0.95	3	0.06	1.02
Air/N_2	..	..	2.7	..	9.9	3.2

low oxygen concentration in the two classes of mutant kernels. The whole losses show only 2.7-fold protection while the mosaics show 9.9-fold protection. This difference is significant at the 2% level ($\chi^2 = 5.7$) as determined by the likelihood ratio test and may offer a clue to the mechanism of oxygen protection. If reduced oxygen tension acts only to reduce the number of chromosome breaks, as has been postulated, there should be no difference in the direction found in the ratio of whole losses to mosaics when the exposures are made in oxygen or nitrogen. That a difference of this kind does exist suggests that it is the mechanism of chromosome restitution following breakage which is sensitive to the oxygen tension in the cell at the time of irradiation.

This will be clarified by an analysis of the types of chromosomal aberrations which give rise to the two classes of mutant kernels. Let us consider first breaks limited to the long arm of chromosome 3. The mosaics arise following a break distal to the *A-Sh* region. After chromosomal duplication the two broken ends of the sister strands fuse in endosperm tissue. Due to the chromatid type of breakage-fusion-bridge cycle a mosaic phenotype

results in the endosperm.[9] The whole-loss kernels can arise from two types of aberrations, (1) chromosome breakage proximal to the *A-Sh* region resulting in a terminal deletion which includes the *A* and *Sh* genes and (2) chromosome breakage on both sides of the *A-Sh* region followed by recombination of broken ends in such a way as to result in an interstitial deletion. It is apparent that the mosaic class results from chromosome breakage without reunion (with the exception of a few rings and dicentrics), while a portion of the whole-loss class (i.e., interstitial deletions) results from breakage with reunion of broken ends. If irradiation in nitrogen increases the ability of broken chromosome ends to rejoin, the number of terminal deletions, and hence dicentric chromatids, would be reduced but the interstitial deletions would not be reduced, since they represent a class where chromosomal rejoining had, in fact, occurred. This would result in a more marked protection of low oxygen tension against the mosaic class as compared to the whole losses.

Mosaic kernels may also arise from other aberrations such as centric rings and dicentrics produced by the fusion of the broken ends of two centric chromosomes. These aberrations produce the chromosomal type of breakage-fusion-bridge cycle.[10] In this study it was not possible to distinguish between endosperm mosaics due to the chromatid and chromosome types of cycles. The frequency of occurrence of rings and dicentric chromosomes which contain the *A-Sh* region was determined in another set of experiments involving sporophyte tissue. The marker used in these studies was the minute, cytologically undetectable $a\text{-}x_1$ deficiency found by Stadler and Roman which includes the *A*, *Sh*, and *W* (a chlorophyll gene) loci.[8] The chromatid cycle persists only in the gametophyte and endosperm while the chromosomal type continues in the sporophyte or plant tissue as well.[10] Hence, plants mosaic for anthocyanin and chlorophyll pigmentation must be due to the chromosomal breakage-fusion-bridge cycle. It was found that a dose of 1000 r in air yielded 4 mosaic plants in a population of 5718. Assuming that the frequency of occurrence of ring and dicentric chromosomes is the same in the endosperm and the sporophyte 4 of the 40 endosperm mosaics obtained in the previous set of experiments should be due to these aberrations and 36 to terminal deletions. On the hypothesis that the oxygen effect is exerted on the recovery process, the kernels which are mosaic because of ring and dicentric chromosomes, would not be protected. Hence, the frequency of their occurrence should not be reduced, but perhaps increased, in the nitrogen treatment. Three such mosaics would be expected out of the 4505 kernels in the nitrogen-treated material. This would account for all the mosaics actually found. Thus, there appears to be an even more striking reduction in the occurrence of terminal deletions than the data actually show.

Breakage involving other chromosomes in addition to chromosome 3 does not affect this picture. Let us consider a case of breakage involving the distal region of chromosome 3 and another chromosome. Reunion of the two centric fragments to form a dicentric chromosome is infrequent and has already been considered. Rejoining of the chromosome 3 centric fragment with either acentric fragment results in a phenotypically normal kernel. Only those cases where the broken end of the No. 3 centric fragment remains free result in mosaics. Hence here, also, mosaics result from breakage without reunion. Where the break in chromosome 3 occurred in the region proximal to the *A-Sh* loci only those cases where the acentric No. 3 fragment failed to rejoin with either centric fragment give rise to whole losses. This class of whole losses is reduced in nitrogen treatment as are the mosaics.

Further work is in progress utilizing a chromosome marked throughout its length, which will facilitate distinguishing between interstitial and terminal deletions.

If oxygen is acting on the recovery process it must be an immediate effect, induced at the time of irradiation, and which persists once it is initiated. Giles and Riley[3] have shown the oxygen tension existing in the cell at the time of irradiation to be the important factor. Altering the oxygen tension immediately after irradiation does not alter the frequencies of chromosomal aberrations. Thus it must be postulated that there is a qualitative difference between broken ends of chromosomes, depending upon the amount of oxygen present at the time of breakage. Chromosomes broken in the absence of oxygen are more capable of rejoining than chromosomes broken in the presence of oxygen. McClintock has presented evidence that the behavior of broken ends is "probably related to the method by which the chromosome becomes broken and to physiological conditions surrounding the broken end."[9]

The data presented are not in agreement with the alternative hypothesis that fewer breaks are produced by irradiation in nitrogen. If this were the case the frequency of mosaics to whole losses should be higher in nitrogen than in oxygen. A reduction in the number of breaks would lessen the probability of the occurrence of two breaks in a single chromosome arm, thereby decreasing the frequency of interstitial deletions and hence whole losses. This is the reverse of what has actually been found.

Summary.—Experiments performed on the irradiation of pollen in air and 100% nitrogen atmospheres have demonstrated the effect of low oxygen concentration on the radiosensitivity of maize chromosomes. The differential protection of low oxygen tension against interstitial and terminal deletions suggests that the effect of oxygen is exerted on the recovery process rather than on the initial breakage mechanism.

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[1] Thoday, J. M., and Reed, J., *Nature*, **160**, 608 (1947).
[2] Giles, N. H., Jr., and Riley, H. P., these PROCEEDINGS, **35**, 640 (1949).
[3] Giles, N. H., Jr., and Riley, H. P., *Ibid.*, **36**, 337 (1950).
[4] Giles, N. H., Jr., and Beatty, A. V., *Science*, **112**, 643 (1950).
[5] Baker, W. K., and Sgourakis, E., these PROCEEDINGS, **36**, 176 (1950).
[6] Hayden, B., and Smith, L., *Genetics*, **34**, 26 (1949).
[7] Mains, E. B., *J. Heredity*, **40**, 21 (1949).
[8] Stadler, L. J., and Roman, H., *Genetics*, **33**, 273 (1948).
[9] McClintock, B., *Ibid.*, **26**, 234 (1941).
[10] McClintock, B., these PROCEEDINGS, **28**, 458 (1942).

NEW WHITE DWARFS IN THE SOUTHERN HEMISPHERE: SECOND LIST

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Following up the color survey of proper motion stars in the Southern Hemisphere reported on previously,[1] results have now become available for the zone with declination limits $-55°$ and $-60°$. In the original survey for the detection of proper motions 48 pairs of plates taken with the Harvard 24-inch Bruce telescope were used. These were blinked by the writer and some 6500 stars were found to possess appreciable proper motion. These motions have all been published in part C of the General Catalogue of Proper Motions. Plates were taken for all 48 regions with the same telescope and on a yellow-sensitive emulsion; these have now been compared with the original blue plates and approximate colors for the 6500 proper motion stars determined.

The vast majority of these stars are ordinary red dwarfs, of course, but some 250 stars were singled out as definitely bluer than expected. Narrowing down the limits still further, forty-three stars have been selected from among these 250 as holding out the greatest probability of being genuine white dwarfs. Data for these stars are given in table 1, the several columns of which require no further explanation.

The forty-three stars have been arranged in four groups, as follows:

(*a*) The first group contains the five stars which had previously been identified as white dwarfs from color plates taken at the Cordoba Observa-