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*THE PRODUCTION OF TWO CHEMICALLY DIFFERENT TYPES OF CHROMOSOMAL BREAKS BY IONIZING RADIATIONS**

BY SHELDON WOLFF AND HENRY E. LUIPPOLD

BIOLOGY DIVISION, OAK RIDGE NATIONAL LABORATORY, OAK RIDGE, TENNESSEE

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The production of chromosome aberrations by ionizing radiations has been studied extensively, mainly because it is a particularly fruitful source of information on the quantitative relations between radiation dose and cellular damage. Primarily, radiation produces chromosomal breaks in direct proportion to the dose administered.[1] Ninety-five per cent of these breaks, as calculated by Lea[2] for *Tradescantia*, restitute, i.e., rejoin in their original configuration. The others may stay open as one-hit aberrations or rejoin with still other broken ends to form two-hit aberrations. These aberrations can be formed only if the two breaks are open simultaneously and in close proximity. If the radiation is administered at low intensity, thus allowing some of the breaks to restitute before others are produced, then fewer two-hit aberrations result than if the same dose were administered at higher intensity.[3]

Intensity studies of this nature in *Tradescantia* led Catcheside, Lea, and Thoday[4] to propose that two types of breaks are produced by the radiation: one group that

rejoins relatively rapidly and another that does so more slowly. Studies on the combined effects of near infrared and X-rays led Kaufmann and Wilson[5] to a similar proposal for *Drosophila* chromosomes.

Previous intensity experiments on the seed of *Vicia faba* indicated that X-ray-induced chromosome breaks stay open for at least two hours when the seed has been soaked in water.[6] However, breaks produced either in BAL[7] or *in vacuo*[8] remain open for only one-half hour. Wolff and Luippold[8,9] have postulated that since this type of break stays open for long periods of time and since respiration and ATP are necessary for its rejoining, it is the break of a strong, possibly covalent, bond. However, Mazia,[10] Steffensen,[11] and Levine[12] have reported that chromosomes are held together by the ionic bonds formed by the divalent cations of calcium and magnesium, and Steffensen[11] suggested that radiation breaks these bonds. Since breaks of ionic bonds cannot be expected to stay open for long periods of time, it is extremely unlikely that any of this type were observed in previous experiments with *Vicia*. The present experiments were therefore designed to ascertain whether or not another type of break existed which rejoined at a faster rate than did those previously described. Such short-lived breaks are indeed found, and their response to the chelating agent Versene suggests that they may be breaks of ionic bonds.

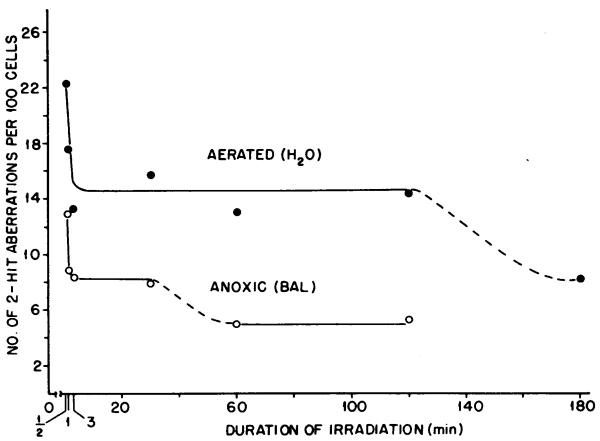

FIG. 1.—Two-hit aberrations produced by 600 r at different intensities.

Seeds of *V. faba* were soaked in distilled water for 24 hours and then peeled and arranged in a culture dish with their embryos facing upward. The dishes were exposed to 600 r of X-rays. For irradiation under anoxic conditions, the seeds were transferred to a 2×10^{-3} *M* solution of BAL one-half hour before exposure. In those final experiments that combined treatments with Versene and radiation, a different crop of seeds was used. Their response to radiation was the same as the crop used in the previous experiments if the seed coats were chipped and the seeds were soaked for only five hours. All irradiations were performed with a G.E. Maxitron tube operated at 250 kvp. with 3 mm. of Al filtration added (hvl., 0.4 mm. of Cu). The seeds were germinated as previously described[13] and fixed in C. E. Ford's modification of Flemming fixative (unpublished). Feulgen squashes were made, and at each point 300–500 metaphases of the first mitotic root-tip division were scored for rings and dicentrics.

The intensity curves in Figure 1 are characterized by plateaus that represent the intervals within which variation of the exposure time has no effect on aberration yield. These plateaus can exist only for times within which no appreciable rejoining occurs, for, otherwise, fewer open breaks would coexist at low intensities

than at high and the number of two-hit aberrations would be correspondingly altered. The length of the plateau wherein there is no diminution of two-hit aberrations is therefore a measure of the time the breaks stay open. This time, which is dose-dependent,[7] is at least two hours in seeds irradiated in water and one-half hour in those irradiated in BAL. If the duration of treatment exceeds these times, rejoining occurs and the typical intensity effect results. However, at radiation intensities higher than 200 r per minute, the number of aberrations produced in air (water) and under anoxia (BAL) increases above the plateau level. This indicates the presence of another type of break that rejoins (either restitutes or forms a two-hit aberration) very rapidly. If the radiation is administered at a very high intensity, many short-lived breaks are present in the cell simultaneously and are capable of yielding many two-hit aberrations. In seeds irradiated with the same dose, but at lower intensity, these breaks usually restitute, and only those that do not rejoin for a relatively long period of time remain. The latter are the breaks, described in previous reports from this laboratory, that are responsible for the plateau on the intensity curves, and are probably of a covalent nature. Thus two sharply distinct classes of breaks can be recognized, one with a lifetime of only about one minute and another that persists from one-half to two hours.

The very rapid rejoining of breaks observed only at the high intensities is consistent with the hypothesis that they are breaks of ionic bonds. For a further test of this hypothesis, seeds were soaked for varying times in a 0.001 *M* solution of ethylenediamine tetra-acetic acid (Versene). This concentration was chosen because it was effective in the studies of Mazia[10] on *Drosophila* and *Melanoplus* that led him to postulate that the chromosome consists of small units bound together by calcium and/or magnesium ions. Table I shows that low concentrations of Versene can break the chromosomes of *Vicia*, too. This can be interpreted as supporting evidence, in plant material, for Mazia's and Steffensen's models of the chromosome.

TABLE 1

PERCENTAGE OF ABERRATIONS PRODUCED BY SOAKING SEED IN VERSENE (0.001 *M*)

Total Cells	Time in Versene (Hours)	No. Aberrant Cells	Aberrations (Per Cent)
650	0	6	0.94 ± 0.38
750	3	27	3.60 ± 0.69
250	6	36	14.40 ± 2.4

We then reasoned that, since a three-hour soaking in Versene produces some chromosome breakage, this period must be long enough to insure penetration of the chelating agent into the cell. If subsequent irradiation at low intensities then produced "ionic" breaks in the chromosomes at the locations of calcium and/or magnesium ions, chelation would occur and the breaks, instead of restituting very rapidly, would stay open to become available for the production of aberrations. The results of a typical experiment of this type are reported in Table 2, which shows that pre-irradiation treatment with Versene in this instance gave a twofold increase in total aberration yield. It should be mentioned that the results of this type of experiment are unusually variable, both between slides and between experiments. Thus, although the increased aberration yield is qualitatively certain, the quantitative results may be poorly reproducible.

Steffensen's earlier observations[11] that the amount of chromosome breakage produced by combined calcium deficiency and radiation is more than the additive amount of both is consistent with these results. The same rationale used to interpret the Versene-radiation effect can also be used to interpret the calcium deficiency–radiation effect.

TABLE 2

PERCENTAGE ABERRATIONS PRODUCED BY COMBINED VERSENE (0.001 *M*) AND RADIATION TREATMENTS*

Slide No.	Treatment	Total Cells	Percentage Normal Cells	Percentage Fragments	Percentage Dicentrics and Rings
1	Versene, 3 hours	50	96.0	2.0	2.0
2		50	100.0	0.0	0.0
3		50	98.0	2.0	0.0
Total		150	98.0	1.33	0.67
1	600 r	50	66.0	24.0	16.0
2		50	80.0	12.0	10.0
3		50	80.0	8.0	12.0
4		50	78.0	12.0	10.0
5		44	72.7	9.1	13.6
6		56	71.5	16.6	17.85
Total		300	74.6	13.6	13.3
1	Versene 3 hours plus 600 r	50	44.0	44.0	32.0
2		50	46.0	44.0	32.0
3		50	58.0	28.0	34.0
4		50	64.0	22.0	16.0
5		50	64.0	22.0	18.0
6		50	82.0	8.0	12.0
Total		300	60.3	28.0	24.0

* Seeds soaked for a total of 5 hours.

It is possible, therefore, to interpret our results on the basis of two chemically different types of chromosome break produced by radiation. One type is a break of covalent bonds that require energy to close. The evidence accumulated here about the other type, although not conclusive, is consistent with the hypothesis that it is the break of an ionic bond.

It is interesting to note that the shapes of the *Vicia* intensity curves, after incorporation of the high-intensity points, are also reflected in the curves obtained when *Drosophila* sperm are irradiated. Haas *et al.*[14] have reported an increased number of translocations from irradiation at very high intensities. These translocations are analogous to the aberrations formed from "ionic" breaks in *Vicia*. These breaks disappear rapidly from the system by restitution, which is the reason they were never observed in previous experiments at relatively low intensities. There is no further intensity effect, because the other breaks, presumably of covalent bonds, stay open until the time of fertilization. These, then, are the equivalent of the breaks that give rise to the plateau of the *Vicia* curves. Since sperm respire very little, the breaks cannot rejoin until they are supplied with the requisite energy produced by respiration of the fertilized egg.

Thus in *Drosophila* sperm it may be postulated that the same two kinds of chromosome break exist and that the length of the plateau is determined not by the recovery of a "rejoining system" as in *Vicia*[9] but by the availability of the energy produced in the egg.

Summary.—Experiments on the seed of *V. faba* indicate that radiation-induced chromosome breaks may have two chemically different natures. One seems to be an ionic break, which closes very rapidly, as would be expected if only electrical factors were necessary for its rejoining. The other is covalent, and it stays open for long periods of time and needs a source of energy for the biosynthesis of the bonds formed in its closing. Such apparently unrelated experimental results as those obtained by the use of calcium deficiency,[11, 12] chelating agents,[10, 12] metabolic inhibitors,[8, 9] and high-intensity radiation[14] can be united by this concept of two types of chromosomal damage.

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*FREQUENCY OF SOMATIC MUTATION TO SELF COLOR IN MAIZE PLANTS HOMOZYGOUS AND HETEROZYGOUS FOR VARIEGATED PERICARP**

BY DONALD R. WOOD† AND R. ALEXANDER BRINK

COLORADO AGRICULTURAL AND MECHANICAL COLLEGE AND UNIVERSITY OF WISCONSIN

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Introduction.—Maize plants heterozygous for variegated pericarp and cob (P^{vv}) and a stable allele (e.g., P^{wr}, colorless pericarp and red cob) bear ears which are more heavily striped than those from homozygous variegateds. The basis of the difference is the number of mutations of P^{vv} to P^{rr} (self colored pericarp and cob) in pericarp tissue. Emerson[1, 2] who first established these facts, estimated that the single P^{vv} allele in $P^{vv}P^{wr}$ plants mutated to P^{rr} about 2.8 times as frequently as each of the P^{vv} alleles in variegated homozygotes. Tests which we and our colleagues have made with P^{vv} alleles of various geographic origins and on several genetic backgrounds have revealed that the numerical relationship differs in different backgrounds and, rarely, may even be reversed. Frequently, however, the difference is comparable to that which Emerson observed.

It will be shown in the present study that the difference in number of mutations