Reprinted from British Science News, Vol. 3, No. 27, pages 66-69.

OXYGEN AND CHROMOSOME MUTATION IN PLANTS

By John M. Thoday, B.Sc., Ph.D.
Department of Botany, Sheffield University.

Introduction

As a tool for the study of mutation ionizin gradiation has, particular advantage, for it is possible to say, rather precisely, how two radiation treatments differ with respect to the number of ionizing particles produced in the cell, their distribution both in space and in time, and the distribution of ionizations left by these particles in their passage across the cell. It therefore seems likely that comparison of the mutagenic effectiveness of different radiation treatments may provide valuable information concerning the precise origin of the changes that result.

From a more immediately practical viewpoint, also, it is important that we should have some comprehensive understanding of the biological effects of radiations, for such understanding is necessary if we are to guard against their damaging effects and if we are to increase their efficiency in the control of malignant growths.

The effects of ionizing radiations (β-, γ-, X-rays, neutrons, and α-particles) on cells are manifold. They may, for example, inhibit enzymes, break down proteins, nucleic acids and nucleo-proteins, affect protoplasmic viscosity, inhibit the progress of cell division, induce gene mutations, and produce chromosome structural changes. It is with the last of these that this article is concerned, though it is evident that this one effect cannot necessarily be regarded as independent of the others.

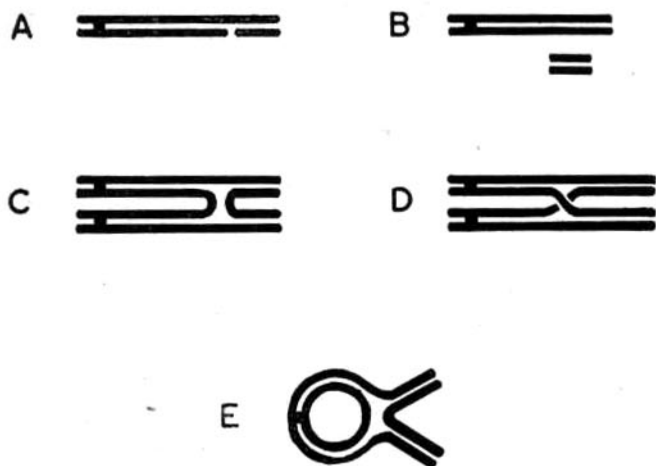

Fig. 1.—Some of the types of structural change as seen in metaphase chromosomes.

A, a chromatid break; B, a chromosome break; C, D, E, different types of chromatid exchange.

These chromosome structural changes are best observed, in plant material, in cells that reach metaphase some time after the irradiation. For the present purpose they may be classified into two broad groups, breaks and exchanges (Fig. 1). Breaks are seen as interruptions in one or both chromatids of a chromosome: two are visible in the cell shown in Fig. 3. Exchanges appear as exchanges of parts between two chromosomes or sections of a chromosome: they evidently involve the production of two breaks and the reunion of these in new ways.

The Target Theory

Although the study of chromosome structural changes began soon after Muller*, in 1927, discovered that X-rays could induce gene mutation, little real advance was made until shortly before the last war when Sax in America published his results obtained with *Tradescantia* pollen grain mitoses. Sax treated inflorescences with X-rays and examined the chromosomes in metaphases fixed 24 hours or 5 days after treatment. Using different doses he found that the frequency of breaks was proportional to the dose used, but that the frequency of exchanges was proportional to some power of the dose greater than unity. If the different doses were administered in the same times this power was approximately 2.

Now an effect that is produced with a frequency proportional to dose may be produced by a unit of dose and, though doses are measured by the number of ionizations they produce, the smallest dose that could theoretically be administered is one ionizing particle, which produces a number of ionizations. It follows that a single ionizing particle can produce a break. But an effect that is produced with a frequency proportional to $(\text{dose})^2$ must require for its production the co-operation of two units of dose. It follows that two ionizing particles are required for the formation of an exchange. Since two breaks are involved in an exchange it is reasonable to suppose that they are independently caused by the two particles involved. Sax confirmed this when he studied the effect of changing the time taken to administer a particular dose. Decrease of dose-rate had no effect on the yield of breaks, but resulted in a decrease of exchanges: the pairs of breaks that exchange may be produced independently, but must be produced near to one another in time. It also follows that some of the "primary breaks" having no partner with which to exchange, restitute: if this were not so, decrease of dose-rate should result in an increase of observed breaks.

Later, Giles in America and the author in England showed, independently, that when neutrons are used instead of X-rays more breaks and exchanges are produced, per

* An alphabetical list of references is given at the end of this article.

ionization. Also the frequency of exchanges, as well as breaks, is directly proportional to dose. When neutrons are used the same number of ionizations are produced in the cells by many fewer ionizing particles than is the case with X-rays and these ionizations are distributed much more densely along the tracks of the particles. There will therefore be a greater probability that the two breaks that exchange shall be produced by the same particle, but only if a particle must pass through or very close to the chromosome if it is to produce a primary break. The linear relation between the frequency of exchanges and neutron dose is therefore evidence that the breaks are produced by particles that pass through or very near to the locus of breakage. That the densely ionizing neutrons produce both breaks and exchanges in greater frequencies, per ionization, than X-rays, shows that more than one ionization is necessary to produce a primary break.

Using these results Lea and Catcheside, in Cambridge, England, have developed Sax's theory and put it on a fully quantitative basis. Starting with the assumption that there is a target volume, associated with the chromosome or chromatid, within which a sufficient number of ionizations must be caused if the probability of primary breakage is to approach unity, and devising methods to calculate the frequency of primary breaks (most of which restitute), they have shown that about 17 ionizations are required in a volume whose diameter is of the order of that of the chromatid, that the half life of a break is about 4 minutes at room temperature, and they have successfully predicted the effects of varying the wavelength of X-rays.

Despite the success of these predictions, the target theory, as it is generally called, has been severely criticized by Darlington and collaborators, who believe that breaks may be produced as a result of disturbances in nucleic acid metabolism. The primary criticism is one of method. The data on which the target theory was based were obtained by comparison of the effects of different treatments on material fixed at constant times after exposure. It is well known that irradiation affects the timing of mitosis. Also, the frequency of aberrations varies considerably with the time that elapses between irradiation and fixation, that is with the stage of the mitotic cycle at which the chromosomes were when irradiated. Therefore, for example, the increase in yield of aberrations with increased dose may be affected by the change in mitotic rate, and thus the dose curves may be distorted.

The Chemical Target Theory

Since the war the author has obtained results that test the importance of this criticism and others that suggest certain developments of the target theory. In the first experiments, *Vicia faba* root tips were exposed to various doses of X- and α-rays (which produce ionizations in fewer, denser tracks, even than neutrons). Roots were then fixed at a number of intervals, ranging from $1\frac{1}{2}$ to 48 hours, after each treatment. Dosage curves were estimated from the means of the frequencies of aberrations for all the "fixation times" so that distortions due to the effects of the treatments on the timing of mitosis might be avoided. Two of the resulting curves are presented in Fig. 2; they have the same shapes as those previously obtained with *Tradescantia*. It seems therefore that the shapes of these curves are not significantly affected by alterations of mitotic rate, and so the target theory stands.

The same experiment has also provided evidence that ionizing particles that do not pass through the target volume may influence the yields of structural changes. Some of the

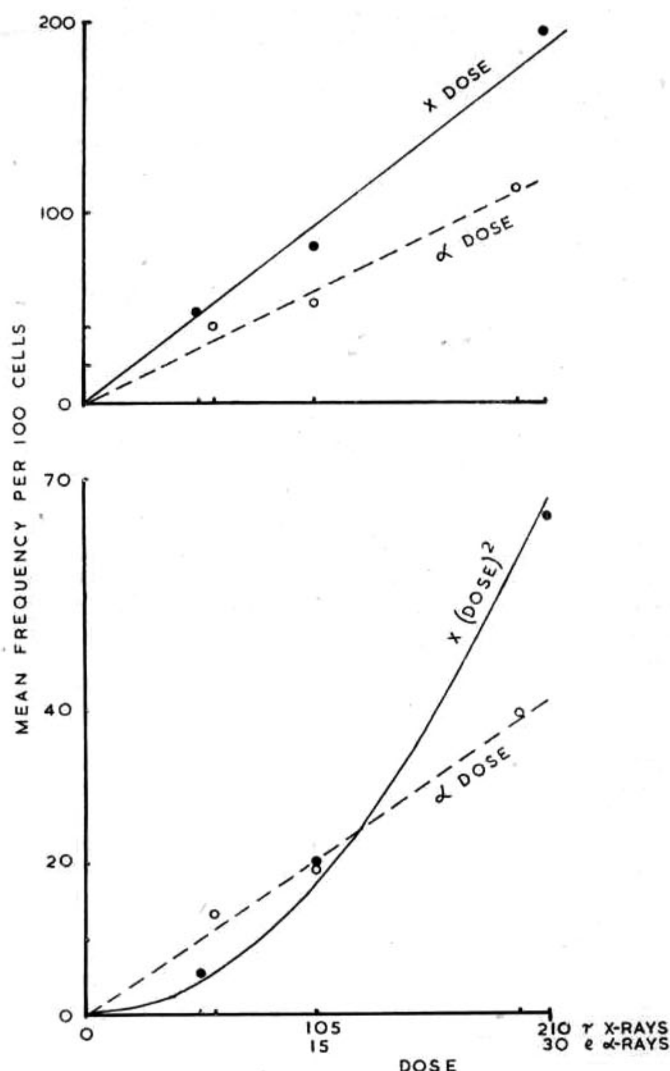

Fig. 2.—The means of the frequencies of chromatid breaks (upper graph), and chromatid exchanges (lower graph), found in Vicia root tip cells.

Cells fixed $1\frac{1}{2}$, 3, 6, 9, 12, 18, 24, 36, and 48 hours after exposure to different doses of X-rays (points), and α-rays (circles). Curves proportional to dose fit all except X-ray exchange data: these are fitted by a (dose)^2 curve. (An e-unit of α-rays is defined as that dose that produces the same amount of ionization in tissue as one r-unit of X-rays.)

exchanges are incomplete: only one of the two possible reunions has been made (Fig. 3). In X-rayed material the proportion of exchanges that are incomplete is not significantly affected by dose and does not vary significantly with fixation time. In α-rayed material on the other hand, the proportion increases as the dose is increased, though this increase is largely confined to the material fixed shortly after irradiation (Table 1). When the number of α-particles is increased some particle, other than that which produces the breaks that exchange, must have some effect that reduces the chance that the breaks will form reunions. But, at the doses used, the chance that two particles shall pass through the same target volume is so small that it can be neglected. The second particle must have been elsewhere in the cell. It follows that irradiation can change the state of the cell in some way that alters the behaviour of the breaks, though the actual production

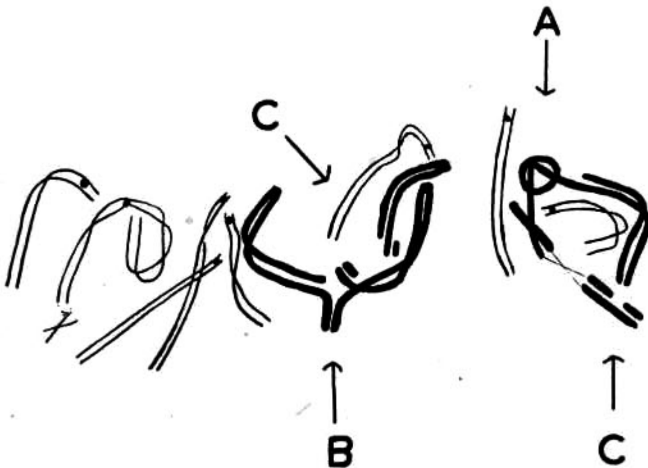

Fig. 3.—Photograph and diagram of a cell showing two incomplete exchanges (photograph by Mr. E. Barron).

A, an incomplete exchange between the two arms of one chromosome (compare Fig. 1 E); B, an incomplete exchange between two chromosomes (compare Fig. 1 D); C, two chromatid breaks.

of the breaks requires, at least in the majority of cases, the passage of a particle through or very near to the locus of breakage.

Table 1.—Ratio of incomplete to complete chromatid exchanges.

Period	Exchanges	Dose		
		$8 \cdot 5e$	$15 \cdot 3e$	$27 \cdot 9e$
1½ to 6 hours	Incomplete exchanges Complete exchanges Ratio	4 21 0·19	15 43 0·35	22 19 1·16
9 to 48 hours	Incomplete exchanges Complete exchanges Ratio	11 35 0·31	16 53 0·30	28 73 0·38
1½ to 48 hours	Ratio of incomplete to complete exchanges	0·27	0·33	0·54

A statistical test shows that the 1½–48 hour ratios differ to an extent that could only occur by chance about once in 400 such experiments (χ^2 is 12·05, there are two degrees of freedom, and the probability of getting χ^2 so large by chance is 0·0025).

The results of the second series of experiments, in which the influence of oxygen on the yields of structural changes has been investigated, must be viewed in this light. The author and Read have shown that the frequency of aberrant cells in *Vicia* is much greater if a particular dose of X-rays is administered in the presence of oxygen than it is if oxygen is replaced by nitrogen. On the other hand, oxygen has little, if any, influence on the effectiveness of α-rays (Fig. 4). The effect of oxygen cannot be metabolic or it would show with both radiations. It parallels closely that of oxygen on the production of H_2O_2 in irradiated water, for Bonet-Maury and Lefort in France have shown that oxygen has no influence on the production of H_2O_2 in water irradiated with α-rays, but that X-rays only produce H_2O_2 if oxygen is present in the water. It therefore seems probable that the influence of oxygen on the production of structural changes indicates that H_2O_2, or some radical that is an intermediate in the production of H_2O_2 from water, is responsible for some of the effects of radiations on the chromosomes.

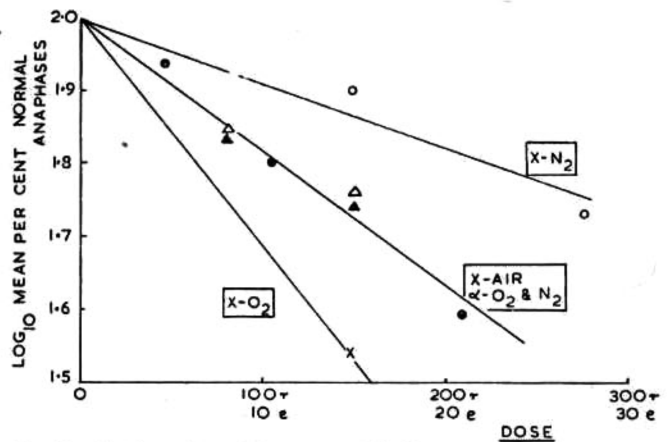

Fig. 4.—The logarithm of the means of the frequencies of "unaffected" cells found in material (Vicia faba) fixed at various times after exposure to different doses of X- and α-rays, with nitrogen, air, or oxygen dissolved in the water in which the roots were treated, plotted against dose. Roots fixed at 3, 6, 12, 24, and 48 hours after exposure.

○ *X-rays, nitrogen.*
● *X-rays, air.*
△ *α-rays, nitrogen.*
▲ *α-rays, oxygen.*
× *X-rays, oxygen.*

This chemical may, in fact, be the agent that is primarily responsible for chromosome breakage. It is, however, also possible that it merely effects some alteration in the physiological state of the cell and thus alters the behaviour of the chromosomes that are broken by some other means. Or it may do both. Further experiment will be required before it can be decided which of these views is correct. But if it turns out that one of these chemicals is the agent responsible for primary breakage, then a considerable step will have been made towards a common theory of radiation mutation, and mutation induced by chemicals such as mustard gas. Radiation would then become a more valuable tool, for it should be possible to determine, from our knowledge of the distributions of ionizations, the distributions of active chemical substances produced in the cell, and to relate these to the genetic effects. This cannot be done with chemical agents introduced into the cell from outside.

Such a chemical theory of the mutagenic action of ionizing radiations would, of course, be perfectly compatible with the target theory. The target theory describes the facts in relation to the distribution of ionizations in columns produced by the ionizing particles. A chemical target theory could equally describe the facts in terms of the columns of active chemical substances produced by the columns of ionizations, and it is not a necessary condition of the target theory that the molecules primarily ionized should be constituent molecules of the gene or chromosome; it is only necessary that the molecules primarily ionized should be situated within the target volume at the time of ionization.

References

BONET-MAURY, P., and LEFORT, M. 1948 Nature (Lond.), **162**, 381.
CATCHESIDE, D. G., and LEA, D. E. 1943 J. Genet., **45**, 186.
DARLINGTON, C. D., and LA COUR, L. F. 1945 J. Genet., **46**, 180.
GILES, N. H. 1940 Proc. Nat. Acad. Sci., **26**, 567.
LEA, D. E., and CATCHESIDE, D. G. 1942 J. Genet., **44**, 216.
MULLER, H. J. 1927 Science, **66**, 84.
SAX, K. 1938 Genetics, **23**, 494.
1939 Proc. Nat. Acad, Sci., **25**, 255.
1940 Genetics, **25**, 41.
THODAY, J. M. 1942 J. Genet., **43**, 189.
THODAY, J. M., and READ, JOHN 1947 Nature (Lond.), **160**, 608.
1949 Nature (Lond.), **163**, 133