EFFECT OF OXYGEN TENSION ON THE INDUCTION OF APPARENT X0 MALES IN DROSOPHILA

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THE production of chromosome rearrangements is the result of two separate processes: the induction of at least two chromosome breaks and the subsequent rejoining of the broken ends in new configurations. It has been possible to study these phenomena independently in *Vicia faba* root-tip mitoses by dose-fractionation experiments (WOLFF and ATWOOD 1954). It was shown that X-rays caused not only chromosome breakage but also a temporary inhibition of rejoining, the extent of which is dose dependent. This inhibition of rejoining is interpreted as damage to a rejoining system (WOLFF and LUIPPOLD 1955) and is seen as an increase in the length of time that breaks produced by one dose remain available to interact, in forming aberrations, with breaks produced by a subsequent dose.

Irradiation under conditions of anoxia produces fewer aberrations than irradiation in air (THODAY and READ 1947). This oxygen effect is observed for induced damage both to chromosomes and to the rejoining system, but the damage to the rejoining system is much more sensitive to the oxygen tension at the time of radiation than is chromosome breakage (WOLFF and ATWOOD 1954). This is best illustrated by the observation that, when the doses given in air and in nitrogen are adjusted to produce equal numbers of aberrations, the breaks produced in air remain open much longer than those produced in nitrogen. The length of time that breaks are held open (e.g., by metabolic inhibitors) does not seem to affect the probability that they will participate in the formation of a scorable aberration (WOLFF 1957). Thus, although there is a much more profound effect of oxygen during irradiation on the rejoining system than on chromosome breakage, the rejoining system eventually recovers and the same number of rearrangements result as there would have been had the rejoining system been unaffected. Therefore, the decreased number of breaks accounts entirely for the decreased yield of aberrations effected by the removal of oxygen during irradiation.

Throughout the remainder of this paper sensitivity to oxygen tension during irradiation will be referred to simply as oxygen sensitivity.

An oxygen effect has been observed for many types of induced genetic damage in Drosophila. This has been attributed either to a greater amount of restitution of breaks induced in nitrogen (BAKER and VON HALLE 1953) or to a decrease in the number of breaks induced in nitrogen (LÜNING 1954). As the result of a series of experiments using dose-fractionation procedures as had been done with the plant material, LÜNING has revised his earlier view and now postulates that comparable amounts of breakage are induced by irradiation in nitrogen and oxygen

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but that breaks produced in nitrogen are more likely than those produced in oxygen to restitute in the original configuration, producing no scorable result (LÜNING and HANNERZ 1957; LÜNING and SÖDERSTRÖM 1957; LÜNING and HENZE 1957; LÜNING 1958). He bases his hypothesis on the observations that, when a given X-ray dose is fractionated into two exposures, one in nitrogen and one in air, separated by 15 minutes, increasing the proportion of the dose administered in air results in an increase in the observed genetic effect until a dose in air of 1000r has been achieved. Any dose in air in excess of 1000r is no more effective than an equivalent dose in nitrogen, and this is true in the total dose range from 3000 to 6000r. The observation that 1080r in air followed by 2160r in nitrogen produces the same effect as 3240r in air (1080r + 2160r) whereas 6480r (4320r + 2160r) in nitrogen is less effective than 6480r in air demonstrates that 4320r in nitrogen does not saturate the oxygen-sensitive system and thus is less effective than 1080r in air, which is a saturating dose. In other words, irradiation of the oxygen-sensitive system in nitrogen results in a dose reduction factor of at least four over irradiation in air. These results indicate that there is an oxygen-sensitive component of the total radiation-induced damage that is saturated by 1000r at 0.2 atmosphere of oxygen. LÜNING believes that this damage is to the rejoining system and that the damage sustained by the genome itself (e.g., breakage) is oxygen independent. The observed saturation has been attributed to a complete inactivation of the rejoining system.

OSTER (1957) compared the effectiveness of 2800r in producing translocations in mature spermatozoa when the radiation was delivered in nitrogen, air, or pure oxygen. If there is a combination of dose and oxygen tension, e.g., 1000r at 0.2 atmosphere, which is capable of completely saturating the oxygen-sensitive system with damage, then no increase in either dose or oxygen tension should result in any further increase in effect on this system; 2800r in air should be no less effective than 2800r in pure oxygen. OSTER's work, however, demonstrates that radiation in air yields results (6.4 percent) intermediate between those from radiation in nitrogen (2.7 percent) and radiation in oxygen (17.2 percent). Thus, it is clear that postulation of a single oxygen-sensitive system damaged to saturation by 1000r in air leads to expectations that are not fulfilled.

Because of the apparent contradiction between the expectations from LÜNING's model and OSTER's observations and because of the differences between the models postulated to account for the oxygen effect in Vicia root-tip mitoses and Drosophila sperm, we have attempted to increase the resolving power of the dose-fractionation procedure by using an oxygen atmosphere in place of air. Fractionation allows variation of the dose delivered at a particular oxygen tension while a constant total dose, and therefore constant oxygen-insensitive damage, is maintained.

We have observed, in agreement with LÜNING, that as the fraction of the dose delivered in air is increased (the remainder of the dose being delivered in nitrogen) saturation is observed. When oxygen is substituted for air in an otherwise identical procedure, however, there is no evidence of saturation and the genetic effect of increasing the proportion of the dose delivered in oxygen continues to increase until the entire dose is delivered in oxygen.

MATERIALS AND METHODS

In previous experiments (LÜNING and HANNERZ 1957; LÜNING and HENZE 1957; LÜNING 1958) the incidence of *y w sn* sons produced by a cross of irradiated $+/sc^{s}$·Y males by *y w sn* females was scored. These are progeny that have inherited neither an intact X chromosome nor a normal allele of *y* from their fathers. It has been assumed that they represent cases of loss of the irradiated X or Y chromosome and that, furthermore, this loss is the consequence of chromosome breakage. We believe that there are data in the literature that provide reasons for doubting the chromosome loss origin of the *y w sn* males and point more strongly to marker loss primarily from the sc^{s}·Y. BAKER (1955, 1957) studied marker loss from a multiply marked Y chromosome sc^{s}·Y:bw^{+} ($= K^{L}bw^{+}$·bb^{+} $K^{S}ac^{+}y^{+}$, whereas sc^{s}·$Y = y^{+}ac^{+}K^{L}$·$bb^{+}K^{S}$) and observed approximately equal numbers of cases of loss of $y^{+}bw^{+}$ and *of* y^{+} alone. These both would have been scored as *y* males in the present experiments, but only the simultaneous losses of y^{+} and bw^{+} can be explained as chromosome loss. In other crosses he observed that for 31 cases of loss of y^{+} but not bw^{+}, there were 95 cases that carried bb^{+} alone. Thus for a given incidence of y^{+} but not bw^{+} or bb^{+} losses, there are at least an equal number of $y^{+}bw^{+}$ but not bb^{+} losses. The incidence of retention of the bb^{+} marker is therefore compatible with the notion that losses of y^{+} are not accompanied by loss of bb^{+} and therefore are not indicative of loss of a chromosome. We agree with earlier views that the *y* males scored are probably the consequence of chromosome breakage.

In the experiments described in this paper, $+/sc^{s}$·Y males were irradiated but the normal X was derived from Oregon-R rather than Canton-S, which had been used by LÜNING, and the irradiated males were crossed to *y* rather than *y w sn*. The males and females were mated individually in vials and a 48-hour sperm sample was collected. LÜNING's observations were based on 24-hour sperm samples or on two successive 24-hour samples. Finally, the males that he irradiated were more homogeneous with respect to age than ours and his females laid for two successive three-day periods, whereas we collected a single six-day sample of eggs. Since our results agree with his, these differences in procedure do not seem to be important.

The irradiation was performed in a lucite chamber through which various gases (nitrogen, air, or oxygen) were passed. The flies were exposed to a flow of a given gas for 15 minutes before irradiation in that gas. Two separate sets of 12 experiments were performed. All irradiations were administered with a G. E. Maxitron tube operated at 250 kvp with 3 mm of aluminum filtration, HVL 0.43 mm of copper. The dose rate was 1000r/min. The total irradiation given was either 2000 or 4000r administered in two 2000r doses separated by 15 minutes. The half-dose (2000r) experiments were performed as controls so that we might check on any departures from additivity and find the expected values after the combined (4000r) exposure. The regular progeny of all crosses were counted with an electronic fly counter (KEIGHLEY and LEWIS 1959).

RESULTS AND DISCUSSION

The results of these experiments are shown in Table 1. The data in the table are the pooled results from the two separate sets of experiments which did not differ significantly from one another. It may be seen that repetition of LÜNING's fractionation procedure, in which he used combinations of air and nitrogen, leads to repetition of his results. The effect of the total dose administered in air was greater than that when the total dose was given in nitrogen (see Table 1. Experiments 2 and 7; 0.826% *vs.* 0.492%), whereas administration of half the total in air and half in nitrogen yielded results comparable to those obtained when the total dose was delivered in air (see Table 1, Experiments 2 and 4; 0.826% *vs.* 0.863%). However, when the difference in oxygen tension between the treatments being compared was increased by replacing air with pure oxygen in the above regime, the results from half the dose in oxygen folowed by half the dose in nitrogen were intermediate between those from the total dose administered in nitrogen or in oxygen (see Table 1, Experiments 7, 11, and 9; 0.492% *vs.* 1.333% *vs.* 2.359%). These findings are presented graphically in Figure 1. It might be noted that combined treatments yield the sum of the individual fractions, indicating a linear dose response.

Although the genetic nature of the radiation-induced effect scored in the experiments described is not thoroughly understood, some generalizations can be made about the damage incurred. There is a fraction of the total damage that is attributable to an oxygen-sensitive system, some component of which (other than radiation dose) may become limiting. We may schematically represent this system as follows:

$$A \xrightarrow{\text{X-ray}} A^* + O_2 \longrightarrow \text{Damage}$$

TABLE 1

The effect of oxygen tension and fractionation on the incidence of y *males from the cross* y *females by irradiated* +/sc^{8}-*Y males*

Expt.	Treatment (min)	Dose I (r)	Treatment (min)	Dose II (r)	No. y ♂/total	Percent y ♂	Percent y ♂ (corrected)*	Expected if additive
1	....	2000, Air	15, Air	....	43/8405	0.511	0.453±0.078	....
2	....	2000, Air	15, Air	2000, Air	66/7460	0.884	0.826±0.109	0.906
3	....	2000, Air	15, N_2	....	47/9530	0.493	0.435±0.072	....
4	....	2000, Air	15, N_2	2000, N_2	46/4992	0.921	0.863±0.136	0.718
5	....	.....	15, N_2	2000, N_2	30/8783	0.341	0.283±0.062	....
6	....	.....	15, O_2	2000, O_2	57/7375	0.772	0.714±0.102	....
7	15, N_2	2000, N_2	15, N_2	2000, N_2	25/4545	0.550	0.492±0.110	0.531
8	15, N_2	2000, N_2	15, N_2	....	29/9466	0.306	0.248±0.057	....
9	15, O_2	2000, O_2	15, O_2	2000, O_2	38/1572	2.417	2.359±0.392	1.946
10	15, O_2	2000, O_2	15, O_2	....	67/6498	1.031	0.973±0.126	....
11	15, O_2	2000, O_2	15, N_2	2000, N_2	45/3236	1.391	1.333±0.207	1.130
12	15, O_2	2000, O_2	15, N_2	.....	63/6960	0.905	0.947±0.114	....
13	Control	.....	....	.....	6/10,303	0.058	0.000	....

* (Observed frequency — control frequency) ± σ.

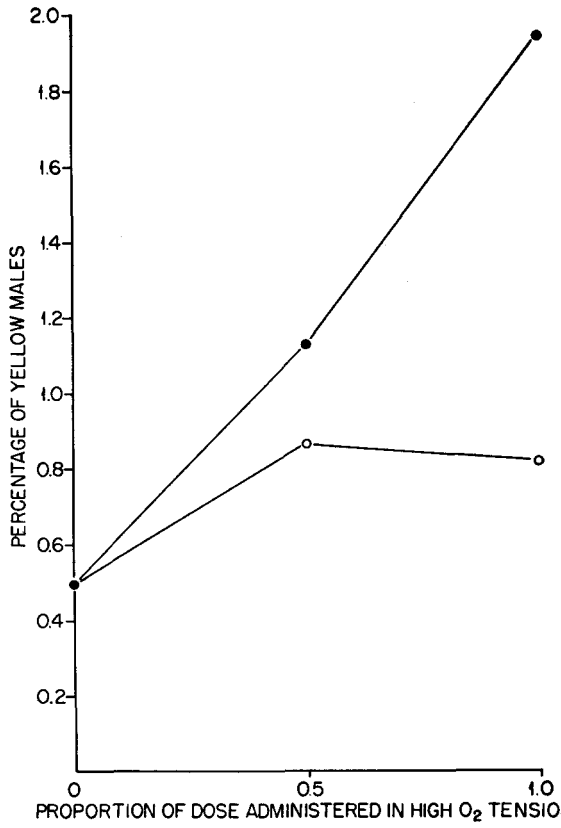


FIGURE 1.—Relation between the genetic effect and the proportion of the dose (4000r) delivered in 0.2 atmosphere (○) or 1.0 atmosphere (●) of oxygen.

where A represents a cellular component that is affected by radiation to yield A*, which in turn reacts with oxygen to yield genetic damage either directly or indirectly. LÜNING has shown when the oxygen tension is 0.2 atomsphere, no further damage to the oxygen-sensitive system can be induced by doses in excess of 1000r. He concludes that one of the cellular constituents (the rejoining system) is limiting and that once it is exhausted no further oxygen-dependent damage is possible. Our results would suggest that this may not be the case since increasing the oxygen tension to 1.0 atmosphere can increase the oxygen-dependent damage above that observed from 1000r at 0.2 atmosphere. From this observation it is possible to argue that oxygen rather than a cellular constituent is the limiting component (cf. POWERS, WEBB, and EHRET VTFJ); however, a plausible alternative is that we are observing the combined effects of several oxygen-sensitive components of damage with different properties.

For example, it is possible that the results are attributable to two independent systems. The first is sensitive to slight changes in oxygen tension (i.e., 0.0 *vs.* 0.2 atmosphere) and is saturated by relatively low doses of radiation. the second is

relatively insensitive to the difference in oxygen tension between 0.0 and 0.2 atmosphere but exhibits increased sensitivity as the oxygen tension exceeds 0.2. Such a model is similar to the one proposed to account for the effect of oxygen on Vicia root-tip chromosomes, where the relatively oxygen-sensitive system corresponds to the rejoining system and the relatively less-sensitive system to chromosome breakage.

It is not possible to describe the quantitative features of the interaction of oxygen tension and dose on radiation-induced genetic damage from the available data. Earlier experiments have varied the proportion of the total dose given at a particular oxygen tension, but have kept the tension constant. The present experiments have varied both oxygen tension and the proportion of the total dose given in oxygen, but data have been gathered from only three doses and two oxygen tensions. Many combinations of dose and oxygen tension will have to be studied before the shape of the surface, which is a function of oxygen tension, dose, and genetic effect will be known. Only then will it be possible to differentiate between the many alternative explanations of the data.

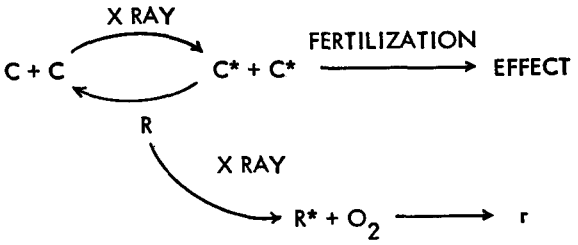
LÜNING (1958) demonstrated that there is also a component of damage that is intensity dependent. The experiment used an initial fraction of 2750r, delivered in nitrogen, in order to protect the postulated oxygen-sensitive repair mechanism, followed by a second fraction of 1650r in air after varying intervals of time. The second dose was sufficient to saturate the oxygen-sensitive system. It was observed that, when the interval between doses was 30 minutes or less, the incidence of y males produced was $\sim$30 percent greater than when the interval was 40 minutes or more. This result has been confirmed for recessive lethals (LÜNING and HENRIKSON 1959). These experiments demonstrate that there is a decay of some radiation product between 30 and 40 minutes after exposure. LÜNING believes that this product is broken chromosome ends. An intensity effect is usually interpreted as evidence that the effect measured results from the interaction of two products of radiation, at least one of which has a limited lifetime. The genetic effect measured by LÜNING shows no deviation from additivity in the present experiments. Therefore, we have an effect that increases linearly with dose and is intensity dependent; it may be represented schematically as follows:

$$A \underset{(R)}{\overset{\text{X-ray}}{\rightleftharpoons}} A^* \longrightarrow \text{Damage}$$

If the damage observed is a function of the concentration of A* produced in the cell, then it is obvious that the equilibrium established will be affected by the rate at which the radiation is administered. If, in addition, there exists a repair system (R) that is damaged by radiation, the equilibrium will be even further shifted in favor of A*.

It is not possible from the available data to determine if this intensity-dependent system and the oxygen-sensitive system are identical.

LÜNING has amended these two generalized schemes into a more restricted picture in terms of chromosome breakage and rejoining. This may be diagrammed as follows:



where R is the rejoining system; r is the inactivated rejoining system; C is the chromosome; and C* is the broken chromosome.

Let us now examine the reasoning that has led to the hypothesis that a rejoining system plays an important role in the preceding scheme. Originally two alternatives were proposed by LÜNING and HANNERZ (1957) as possible explanations of oxygen sensitivity. They were termed differential sensitivity and rejoining. It was postulated that, were chromosome breakage oxygen sensitive (i.e., differential sensitivity), the genetic effect of 3240r administered as either 1620r in air plus 15 minutes in nitrogen plus 1620r in nitrogen or as 1620r in nitrogen plus 15 minutes in air plus 1620r in air should be intermediate between that produced by 3240r in nitrogen and 3240r in air. It was observed, however, that the fractionated treatment yields an incidence of *y* males equivalent to the yield from 3240r in air (LÜNING and HANNERZ 1957; LÜNING and HENZE 1957). Similar observations have been made for sex-linked recessive lethals (LÜNING and SÖDERSTRÖM 1957) and translocations (LÜNING, personal communication). Because the postulated expectations from differential oxygen sensitivity of chromosome breakage were not realized, it was concluded that the observations favored the rejoining hypothesis. It is clear, however, that the terms differential sensitivity and rejoining both refer to oxygen sensitivity—of the chromosomes in the first instance and of the repair mechanism in the second. We believe that, although an intermediate effect of a dose-fractionation regime can be postulated as the expected consequence of the hypothesis that breakage is oxygen sensitive, it can equally well be the expected consequence of the hypothesis that the repair mechanism is the sensitive system. In the latter case, the rejoining hypothesis would have been rejected and differential breakage accepted on the basis of the same observations. In other words, we submit that the peculiar saturating effect of 1000r in air was not an *a priori* expectation for either alternative originally postulated. It should be further pointed out that had oxygen been used in place of air in the original experiments (LÜNING and HANNERZ 1957), the postulated expectation would have been fulfilled and the results would have been interpreted as favoring differential sensitivity not rejoining.

From the foregoing considerations it is clear that there is no justification for using the dose fractionation described in support of the hypothesis that there is

an X-ray-sensitive rejoining system in the mature sperm of Drosophila. Furthermore, since a rejoining system, in order to be in accord with other observations, would have to have the rather bizarre features of effecting restitution but not reunion and of operating on some broken ends but not others, it would seem more logical at this point to attribute oxygen sensitivity to some portion of the original genetic damage itself.

SUMMARY

LÜNING and co-workers have established that in Drosophila sperm there is a peculiar dose-oxygen tension interaction that results in saturation of an oxygen-sensitive system by 1000r delivered at 0.2 atmosphere of oxygen. The present experiments have demonstrated that, when the radiation is delivered at 1.0 atmosphere of oxygen, no saturation of the oxygen-sensitive system is achieved.

The results may be interpreted to indicate that at 0.2 atmosphere of oxygen and 1000r of X-rays, oxygen rather than a cellular component limits the amount of damage that may accrue.

Alternatively, the results are also consistent with the existence of two oxygen-sensitive systems, one of which is exhausted by 1000r at 0.2 atmosphere of oxygen and the other of which is insensitive to the difference between 0 and 0.2 atmosphere but is sensitive to 1.0 *vs.* 0.2 atmosphere of oxygen. This model is similar to the one proposed to account for the effect of oxygen tension on induced chromosome breakage and rejoining in *Vicia faba*, in which a rejoining system is extremely sensitive to oxygen tension, whereas breakage is less sensitive.

Therefore, there are at least two explanations of the phenomenon; however, the available data do not distinguish between them.

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