THE EFFECT OF CARBON MONOXIDE AS A RESPIRATORY INHIBITOR ON THE PRODUCTION OF DOMINANT LETHAL MUTATIONS BY X-RAYS IN DROSOPHILA

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The modifying influence of carbon monoxide on the extent of X-ray-induced genetic damage in Drosophila has been studied in the past by Haas, Dudgeon, Clayton and Stone (1954); Stone, Haas, Alexander and Clayton (1954); Alexander and Stone (1955); Fritz-Niggl (1959); Chang, Wilson and Stone (1959); and Oster (1959). Irradiation, delivered in an environment of carbon monoxide, with or without intentional addition of a few percent of air or oxygen, regularly gave a higher damage than if an inert gas was used instead of carbon monoxide. This refers to the production of dominant and recessive lethal mutations and of translocations. Working with root tip cells of Vicia faba, Kihlman (1958) has reported a great influence of very small amounts of oxygen on X-ray-induced breakage of chromosomes, if respiration of the cells was blocked by respiratory inhibitors. Carbon monoxide is such a respiratory poison, blocking cytochrome oxidase (a light reversible reaction).

During recent years another role of the respiratory enzymes in connection with radiation effects came into the foreground, a role concerning chromosomal reunion processes, restitution and recombination of broken chromosomes. Most of this work has been done with plant material, but it has been shown that these processes also play a role in Drosophila in oocytes and in early spermatids, (Oster 1955; Herskowitz and Abrahamson 1956; Abrahamson 1959; Parker 1959; Falk 1959). It seems to be a well-established fact that chromosomal reunion processes require a supply of energy which normally is dependent on an undisturbed activity of enzymes engaged in respiration. In this connection Beatty and Beatty (1960), using microspores of Tradescantia, published results indicating that carbon monoxide in a mixture with five percent oxygen exerted an influence only on the reunion processes leading to different amount of chromosomal interchanges. In this test, the amount of primary breaks following treatment in carbon monoxide was the same as the amount following treatment in helium plus five percent oxygen. This is in contrast to the findings obtained with Drosophila, where for the production of dominant lethals, carbon monoxide has an enhancing effect if present during irradiation but where no posttreatment effect could be shown (Alexander and Stone 1955). The findings of Kihlman (1958)

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and of Beatty and Beatty (1960) made it desirable to investigate the situation further in Drosophila.

In order to do this, we performed the following experiments:

*Test 1.* To prove or disprove the assumption that previous posttreatment experiments had been too short—Alexander and Stone (1955) had posttreated for 30 minutes—we applied a very long posttreatment of nine hours.

*Test 2.* In order to test the influence of small amounts of oxygen with blocked enzymes, we compared the dominant lethal rate obtained by 1000r in an environment of tank-CO with the rate obtained in CO + 2% O₂ and in He + 3% O₂, at a temperature of 22°C.

*Test 3.* In this test we wanted to see if it was possible to show an effect of the small oxygen impurities which are contained in commercial carbon monoxide. We compared the dominant lethal rate obtained by 1000r in an environment of carbon monoxide (indicated minimum purity: 99.5 percent with the rate obtained in helium of a minimum purity of 99.99 percent, the irradiation being done at a temperature near the freezing point in order to eliminate enzyme activity in both cases.

**MATERIALS AND METHODS**

Young males of *Drosophila virilis*, Mexico strain (Texmelucan), were irradiated at an age of 12–24 hours. Afterwards they were aged for five days (adult males of *D. virilis* require six days to mature) and then were mated individually to three virgin females from a cross of "Argentina strain" × "Brazil strain" in order to obtain maximum heterosis. Every two days for a period of from ten to 18 days the males were remated to three new females. From each mating one or two females were separated, and all eggs laid on the second, third and fourth days after the mating period were counted. The percentage of dominant lethal mutations was determined from the proportion of eggs which failed to develop into pupae.

The X-rays were obtained from a constant potential machine (250 kv; 15 ma; filter: 0.5 mm Cu + 1 mm Al). The dose rate was 330r/min and the doses were measured with a Victoreen-r-meter. The flies were irradiated always in the same plexiglass container of 22 mm diameter and a height of 5 mm which could be closed gas-tight. The desired gas was administered to the flies through an inlet and an outlet in the bottom. In each series 50 males were treated simultaneously.

In test 1a (CO-posttreatment) and 1b (control), irradiation with 500r was performed in air at a temperature of 22°C. The flies which were posttreated with carbon monoxide were transferred to a larger container and exposed to a mixture of 95% CO + 5% O₂ within 60 seconds after irradiation. Under this treatment the flies remain completely motionless. They were stored, together with the control group in air, in the dark for nine hours.

In tests 2a, 2b, and 2c, X-ray doses of 1000r were given at 22°C. In 2b, tank-CO was flooded through the container for six minutes, and the flies were held in that gas for an additional six minutes before irradiation. In test 2a, 120 cc of a gas
mixture of tank-CO + 2% O₂ were flooded through the container before it was closed; the flies were held in this mixture for 12 minutes. The pretreatment and the irradiation were given in both cases in the dark. The containers were opened four minutes after irradiation. In test 2c, 120 cc of the gas mixture of He + 3% O₂ were used, but the pretreatment in this case was reduced to two minutes in order to prevent a significant amount of oxygen consumption before irradiation.

In test 3, irradiation with 1000r was done (3b) in helium (indicated purity 99.99 percent minimum) and (3a) in tank-CO (purity 99.5 percent minimum). The respective gas was flooded through the container for two minutes; then the container was submerged in ice water for 12 minutes after which the irradiation dose was immediately administered in a precooled environment and in the dark. The containers were opened four minutes after irradiation.

For the purpose of comparison it is advantageous to plot the obtained values in a curve, although one must be aware that the connecting lines have no meaning but are just a visual help. In our nomenclature “A” means sperm irradiated 5–7 days before fertilization, “B”, 7–9 days and so on. It is not possible to attach all these letters to a certain stage of spermatogenesis, because the different treat-

<table>
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<th>TABLE 1</th>
<th>The frequency of dominant lethal damage in Drosophila virilis in different stages of spermatogenesis after irradiation under various conditions. See text and Figures 1–3 for other information</th>
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ments sometimes have a different influence on the speed of spermatogenesis. It is therefore necessary to look at the curve as a whole and not to put too much emphasis on the values obtained in a single mating period.

RESULTS

In test 1, (Table 1, Figure 1) nine hours of posttreatment with carbon monoxide did not influence significantly the dominant lethal rate obtained after a dose of 500r in air. The whole curve la is slightly shifted to the right side, due to a delay in the spermatogenesis of the CO-posttreated males.

*Figure 1.*—The frequency of dominant lethals in *Drosophila virilis* plotted for the different stages of spermatogenesis, after an irradiation with 500r X-rays at 22°C. 1a: nine hours of posttreatment with carbon monoxide; 1b: no posttreatment. A: mating period 5–7 days after irradiation, B: 7–9 days, etc.

*Figure 2.*—The frequency of dominant lethals in *Drosophila virilis* plotted for the different stages of spermatogenesis after an irradiation with 1000r X-rays at 22°C in an environment of the indicated gases.
The frequency of dominant lethals in *Drosophila virilis* plotted for the different stages of spermatogenesis after an irradiation with 1000r X-rays at 0–2°C in an environment of 3a: CO, 99.5 percent pure and 3b: He, 99.99 percent pure.

The result of test 2, (Table 1, Figure 2) is impressive. The difference in the amount of dominant lethal damage caused by the addition of 2% O₂ to the carbon monoxide is very marked. In the sensitive stages of spermatogenesis, in spermatids and spermatocytes, which are represented in the upper part of the curves (Figure 2), over 50 percent more dominant lethal damage is observed in CO + 2% O₂ than in CO alone. The difference dwindles when adult sperm are involved (left end of curve); it is well known, however, that changes in environmental conditions during irradiation have little influence on mature sperm. As a whole the differences are similar to the ones obtained by 1000r and 500r in air with the same dominant lethal system (Alexander, Bergendahl and Brittain 1959). Curve 2c (He + 3% O₂) is very similar to 2b (CO), with the exception that the more immature stages are shifted to the left due to the absence of the delaying effect of CO on the speed of spermatogenesis. Curve 2a falls into the range of the results obtained by irradiation with 1000r in air, and the curves 2b and 2c are slightly above the values obtained by the same dose in pure inert gases.

The results of test 3, (Table 1, Figure 3) show (3a) that if the purest degree of commercially available CO (99.5 percent) is used under great care to keep it pure, the effect is a good deal smaller than was usually claimed. On the other hand the comparison with 99.99 percent pure helium (3b) shows that even less than one half percent oxygen present in a gas has a small but clear-cut effect, provided respiration is blocked.

**DISCUSSION**

The dominant lethal method, in the form in which it is used for these experiments, serves as a good measure of genetic damage in *Drosophila*. It has been known for several years that spermatids and spermatocytes are much more sensitive to changes of the environmental conditions during irradiation than are
sperm. But, due to the simultaneous influence of the treatments on the speed of spermatogenesis, the results seemed to be very unreliable if one tried to compare the results obtained only at a single point of the curve, that is, at a certain number of days after irradiation. Comparisons using spermatids, therefore, came into an unfavorable light, and sperm, which reacts so poorly, have been the preferred material for a time. A better method is to record the dominant lethal rate obtained in a continuous sequence of mating periods. These entire sequences then can be compared to each other and an eventual shift of the most sensitive stage to an earlier or later mating period is recognizable. This method has been applied with great success especially by M. L. Alexander during the last few years.

Certain difficulties are caused by the occurrence of unfertilized eggs. Most of them can be distinguished easily from dominant lethals if the eggs laid by individual females are counted and recorded separately. If there is no development among large numbers of eggs of a female, or, in exceptional cases, only strikingly few, then this is clearly due to the absence of sperm and such egg counts can be excluded. This is not possible in methods where the egg-laying females are kept together. Unfertilized eggs are a more serious problem than the statistical errors, since the latter become relatively negligible if the values out of which the dominant lethal curves are composed are based on counts of 500–1000 and more eggs.

The kinds of chromosomal breaks that lead to dominant lethals in sperm and spermatids of Drosophila obviously cannot be influenced in the same way as the breaks leading to translocations. Also an extension of the posttreatment with carbon monoxide to nine hours did not bring about additional damage by preventing eventual restitution. The reunion processes following isochromatid breaks in part apparently obey other laws. If it were possible to prevent reunions of the broken chromatids in their original arrangement thereby favoring terminal deletions and the occurrence of sister chromatid unions, one might expect an increased number of dominant lethals. The negative results, so far obtained, have always been based on the one energy-dependent mechanism. It might be possible that another kind of interaction based on a different mechanism of reunion could be successful in changing the fate of broken chromosomes.

The finding of Kihlman (1958) that in Vicia faba, if respiration is blocked, small amounts of oxygen cause a strong enhancement of the radiation effect applies also to the production of dominant lethal mutations in Drosophila, as we were able to show in tests 2 and 3. For a long time the view was widely held that small amounts of oxygen could be neglected, based on the observations that, if irradiation is performed in an inert gas (N₂, He) and at room temperature, an addition of up to three percent oxygen shows almost no enhancing effect; this is demonstrated once more in our test 2c. Now, it seems to be most probable that under these circumstances the oxygen is removed by respiration before it reaches the chromosomes and their immediate neighborhood within the nucleus. But this enzymatic removal of oxygen stops as soon as the cellular respiration is blocked by respiratory poisons. Figure 4 is an illustration of that mechanism.

Previous theories about protective functions of the cytochrome system become
Figure 4.—The drawing shows a symbolic cell with mitochondria and the nucleus. If the oxygen supply from outside is limited, respiration at a normal rate removes oxygen before it reaches the nucleus. If the outside oxygen concentration is less than three percent, respiration will remove all oxygen; with five percent oxygen only a very small amount can reach the nucleus. But, when respiration is inhibited, even low concentrations of oxygen gain access to the nucleus and influence chromosome damage by radiation. No quantitative accuracy is intended in this drawing.

superfluous, as is discussed in detail by Kihlman, Merz and Swanson (1957) and Kihlman (1958). It should be mentioned here that Stapleton, Billen and Hollaender (1952) had already described "The role of enzymatic oxygen removal in chemical protection against X-ray inactivation of bacteria." It has taken a long time for all the consequences of the mechanism described in that paper to be recognized and proved for other organisms.

SUMMARY

1. Dominant lethal damage in immature germ cells of Drosophila virilis, obtained by X-irradiation in carbon monoxide, is drastically enhanced by the presence of very small amounts of oxygen. This is in accordance with the findings reported for plant material by Kihlman where the same effect was shown for chromosomal damage in the presence of several other respiratory inhibitors. Small amounts of oxygen which would easily be removed by cellular respiration get access to the vicinity of the chromosomes if respiration in the cytoplasm is blocked.

2. Posttreatment with CO + 5% O₂ for nine hours in the dark with the flies immobilized by the CO showed no effect on the X-ray-induced dominant lethal rate obtained from mature and immature stages of male germ cells.

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LITERATURE CITED


