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THE EFFECT OF OXYGEN CONCENTRATION ON THE RATE OF X-RAY INDUCED MUTATIONS IN DROSOPHILA

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Various supplemental agents have been administered to organisms being exposed to X rays and the effects of these agents on the induced frequency of gene mutations and chromosome aberrations have been studied. In general, the supplemental treatments which did alter the X-ray induced mutation or chromosome rearrangement rate were found to increase this rate. However, the recent work of Thoday and Read[1], Hayden and Smith[2], and Giles and Riley[3] provide evidence that by lowering the oxygen tension during X-ray exposure of plant material, the induced frequency of chromosome aberrations is greatly reduced. In view of the implications of this finding, it seemed advisable to determine if a lowered oxygen concentration reduces the radiation damage to the genes in animal material.

Experimental Methods.—The induction of sex-linked, recessive, lethal mutations was investigated in the Oregon-R strain of *Drosophila melanogaster*. Wild-type males of this strain (from one to three days in age) were treated with X rays in the manner described below and then immediately mated in a culture bottle to virgin females of the Muller-5 stock ($sc^{Sl}B$ In-S w^a sc^8)[4]. Seven to eight days after the time of treatment the parents were removed from the culture bottle. The F_1 females, all of which carry an irradiated X chromosome, were aged with their brothers (Muller-5 males) for at least two days in a fresh culture bottle in order to increase the chance of insemination. Approximately the first 200 F_1 females which hatched from the parent culture were separated individually after aging into shell vials containing one or

*Consultant

two Muller-5 males. A preliminary examination was made of the F_2 offspring for the presence of wild-type males by viewing the flies inside the culture vial with a binocular dissecting microscope. If one or more wild-type males were observed, the X chromosome being tested was classified as a chromosome with no lethal mutations. On the other hand, if no wild-type males were observed in the preliminary examination of the F_2 flies, the culture was set aside until all the offspring had hatched and then the flies were anesthetized and classified. If this examination verified the absence of wild-type males, a further test of this lethal-bearing X chromosome was made by mating in a shell vial three of the F_2 females which were heterozygous for the Muller-5 chromosome to five of their Muller-5 brothers. The F_3 offspring were counted to substantiate the presence of a lethal-bearing chromosome.

Because of the intergradation between mutations that have a full lethal effect and those which are semilethal, the classification of a mutation as a lethal mutation is arbitrary. An X chromosome was classified as having one or more lethal mutations if the following criterion was valid: no wild-type males in the F_2 generation and 5% or less males of this type in the F_3. Less than 2% of the F_2 cultures with no wild-type males contained less than twelve Muller-5 males. These were classified as lethals only if 15 or more offspring were present; otherwise, they were classed as failures.

X-radiation was administered to the flies by means of a General Electric, Maximar Model, 250 kvp unit which contained a self-rectifying Coolidge tube with a tungsten target. The inherent filtration was equivalent to 3 mm. of aluminum. In all the experiments 250 kvp X rays, produced by a 15-ma current, were used. This gave a dosage rate of about 125 r per minute at the target distance of 61.5 cm. The dosage rate was kept constant in all experiments.

During irradiation one group of flies was maintained in a continuous flow of oxygen gas and another group in a continuous flow of nitrogen gas; both groups being kept at a known constant temperature by means of the apparatus to be described. The

gases, obtained from commercial cylinders, were passed through copper coils which were immersed in a water bath containing either tap water or a mixture of tap water and ice. A beaker with two pairs of small side arms was also partially immersed in this water bath. The gas, after passing through the copper coils, entered the beaker by means of one of the side arms. Between this side arm and the one on the opposite side of the beaker, was stretched a rubber balloon (open at both ends) which contained the flies enclosed in a gauze capsule. After the gas passed over the flies, it was conducted through copper tubing from the beaker to the outside of the water bath container. Two sets of copper coils and four side arms on the beaker made possible the simultaneous exposure to X rays of one group of flies in oxygen and another group in nitrogen, both groups being maintained at the same temperature. The top of the beaker, which extended just above the surface of the water bath, was covered by a lucite lid and the radiation was administered to the flies through this window. In addition to the four small side arms previously mentioned, there was one large side arm on the beaker which extended from the beaker to the outside of the water bath. This side arm allowed insertion of a thimble chamber into the beaker at the location where the flies received the radiation and also made possible the insertion of a thermometer into the beaker for temperature measurements.

The treatment procedure may be summarized as follows. The irradiation apparatus was placed underneath the target port and the dosage being delivered was determined by placing the thimble chamber into the beaker. The thimble chamber and the electrometer used had recently been standardized. The calculated dosage to be given was obtained by averaging the five dosimeter readings taken. About 150 Oregon-R males were placed in each of two gauze capsules and each capsule inserted into one of the balloons. The gases were then passed across the flies and ten minutes later the temperature inside the beaker was checked and the X-ray exposure begun. After the time necessary for the desired dosage had elapsed, the males were removed from the gas and mated immediately to Muller-5 females.

Experimental Results.—The data were gathered by means of 31 individual experiments of the type described above. Chi-square tests of homogeneity of the three or more experiments conducted with a given gas, temperature, and dosage show no indication of heterogeneity among the individual experiments. Therefore, it is permissible to lump the results from the individual experiments in which the flies received the same treatment. The lumped data showing the effects of oxygen concentration, temperature, and dosage on the frequency of induced lethal mutations are compiled in table 1. The striking reduction in the number of lethal mutations induced when the flies are maintained in a nearly oxygen-free atmosphere is clearly evident from this table and the curves presented in figure 1. Chi-square values obtained by testing the independence of the gas used on the mutation frequency are tabulated in the seventh column of this table. The reduction in the mutation rate in the nitrogen series is highly significant ($P<0.01$) except in the experiments in which the flies were given 1000 r units and kept at the warm temperature. There seems no reason to doubt that more extensive experiments conducted at this dosage and temperature would provide a statistically significant difference. The results assembled in table 1 also furnish direct evidence, which substantiates the claim of some previous investigators[5], that more mutations are induced when the flies are kept at a near freezing temperature than are induced at room temperature. The chi-square values obtained from tests of independence of temperature on mutation rate are tabulated in the last column of table 1. At all three dosage levels more mutations are produced at the lower temperature when the flies are irradiated in an oxygen atmosphere, but the difference is statistically highly significant only in the 3000 r series. In the nitrogen treated flies, on which more extensive data are available at the higher dosages, the temperature difference causes no apparent alteration in the mutation rate. At 5000 r units the chi-square value is on the borderline of the conventional 5% level of significance. These results seem to indicate that the

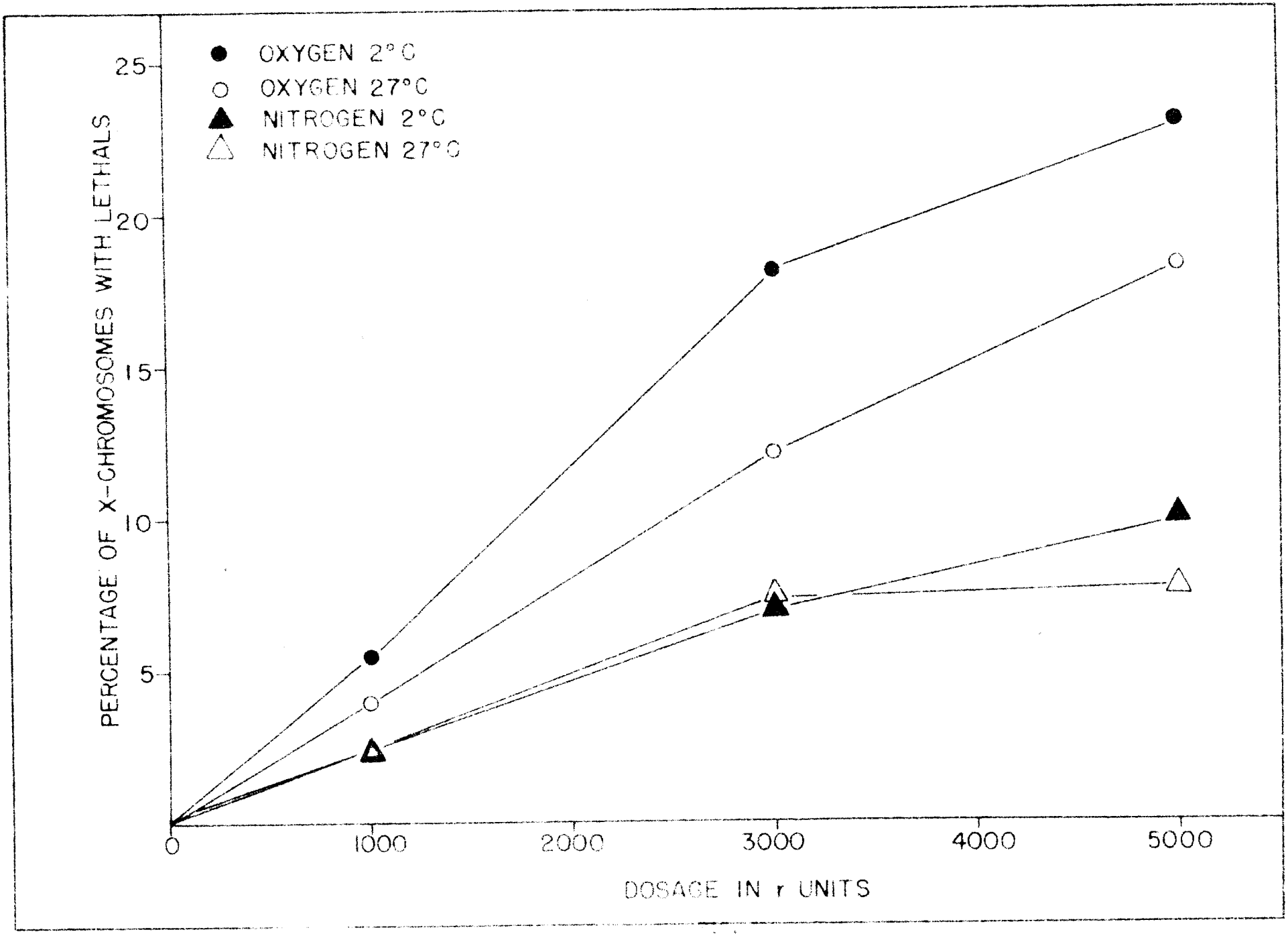
Table 1

EFFECT OF O_2 CONCENTRATION, TEMPERATURE AND DOSAGE ON LETHAL MUTATION RATE

Dosage	Temperature range	Gas	No. chromosomes tested	Number lethals	Percent lethals	χ^2_{gas}	$\chi^2_{temp.}$
Control	0.5 - 1.0	O_2	602	1	0.2	---	---
	0.5 - 1.0	N_2	608	2	0.3		
	26.0 - 28.5	O_2	607	1	0.2	---	---
	26.0 - 28.5	N_2	605	1	0.2		
1000 r	1.5 - 3.0	O_2	600	33	5.5	7.4	O_2 1.6
	1.5 - 3.0	N_2	582	14	2.4		
	22.0 - 28.0	O_2	605	24	4.0	2.5	N_2 0.0
	22.0 - 28.0	N_2	594	14	2.4		
3000 r	1.0 - 3.0	O_2	485	88	18.1	36.4	O_2 7.8
	1.0 - 3.0	N_2	767	54	7.0		
	26.0 - 28.2	O_2	603	73	12.1	9.0	N_2 0.1
	26.0 - 28.2	N_2	775	56	7.2		
5000 r	1.0 - 3.0	O_2	105	24	22.9	40.3	O_2 1.2
	1.0 - 3.0	N_2	1206	119	9.9		
	23.0 - 29.0	O_2	429	78	18.2	16.8	N_2 3.8
	23.0 - 29.0	N_2	1523	118	7.7		

Figure 1

X-ray dosage curves for the lethal mutations induced in *Drosophila* irradiated in oxygen or nitrogen gas at 2° and 27° C.


OXYGEN 2°C
OXYGEN 27°C
NITROGEN 2°C
NITROGEN 27°C
PERCENTAGE OF X-CHROMOSOMES WITH LETHALS
25
20
15
10
5
0
1000
2000
3000
4000
5000
DOSAGE IN r UNITS

"temperature effect" is actually an effect of altered oxygen tension in the sperm possibly caused by differences in oxygen solubility and rate of respiration at the two temperatures. It should be noted that the control experiments conducted without irradiation are not sufficiently large to give any reliable information concerning a possible relation between oxygen tension and spontaneous mutation rate.

It is evident from the figures presented in table 1 that at the higher dosages fewer chromosomes were tested in the oxygen treated flies than in the nitrogen series. Although a large number of experiments were conducted with the flies in oxygen, only a relatively small number of F_1 offspring were produced from the males which had been maintained in this gas during irradiation. The reduced number of offspring in the oxygen as compared to the nitrogen series could possibly be due to any of four factors: (1) reluctance of the males to mate, (2) sperm immotility*, (3) lowered viability of the treated males, or (4) increase in the frequency of dominant mutations and chromosome aberrations which are lethal. Experiments were undertaken to determine which of these factors was effective. After irradiation of the Oregon-R males with 5000 r units at 27°C., sixty matings were made; each mating consisting of three males treated in oxygen and five Muller-5 females placed together in a shell vial. Another 60 similar matings were made using the males which had been exposed in an atmosphere of nitrogen. Immediately after treatment the males in both series were checked to see if any had been killed during irradiation. Every 24 hours after exposure, the number of males living in each culture was observed; also, ten cultures were selected at random in each gas series and the reproductive tracts of the five females in each culture were dissected out and examined for the presence and motility of sperm. This procedure was repeated every 24 hours after treatment for six days. The number of offspring produced by each culture was counted. This gives an estimate of the number of offspring per culture produced from the eggs laid during the first 24, 48, 72, etc., hours after treatment.

*There is a remote possibility that the oxygen gas might affect the ability of the sperm to fertilize the eggs without disturbing their motility.

The smaller number of offspring per culture produced by males which were exposed to 5000 r units of X rays while in oxygen as compared with nitrogen can be seen in figure 2. Since the number of progeny in the two gas series is the same when the parent males are exposed to the gases without radiation, it is obvious that this effect must be caused by a difference in the biological action of the radiation on the flies in the two gases.

Only one case was observed of immotile sperm in the reproductive tract of the female and no evidence was obtained of any difference in the number of females inseminated; between 90 and 100% of the females were inseminated in both series from the first day on. These observations eliminate the possibility that the first two factors listed above are effective. The data presented in figure 3 indicate that male viability is not an important factor since it acts in the opposite direction. About one-half of the males exposed in nitrogen die during the first few days, while the viability of the flies in oxygen is not affected over the course of the experiment. Incidentally, it appears from a comparison of the two nitrogen curves in figure 3 that the differential killing is not only caused by the gas alone but is enhanced by the radiation. These findings then provide evidence that the difference in the number of offspring produced from males treated in the two gases must be attributed to a reduction in the frequency of induced dominant lethal mutations when the flies are irradiated in nitrogen. Further evidence, based on the smaller number of failures of the F_1 matings in the nitrogen series also indicates a lowering of the frequency of dominant sterility mutations in the absence of oxygen.

Discussion.—It appears reasonable to attribute the reduction in the frequency of mutations induced in the male flies irradiated in a nitrogen atmosphere to a lowered oxygen tension in their sperm. It must be assumed then that the nitrogen acts as an inert gas insofar as this effect is concerned and, also, the assumption must be made that, by greatly reducing the oxygen concentration surrounding the flies, the oxygen

Figure 2

Relationship between the oxygen concentration at time of irradiation and the number of offspring produced. On the abscissa is plotted the number of days after treatment during which the females were allowed to deposit eggs.

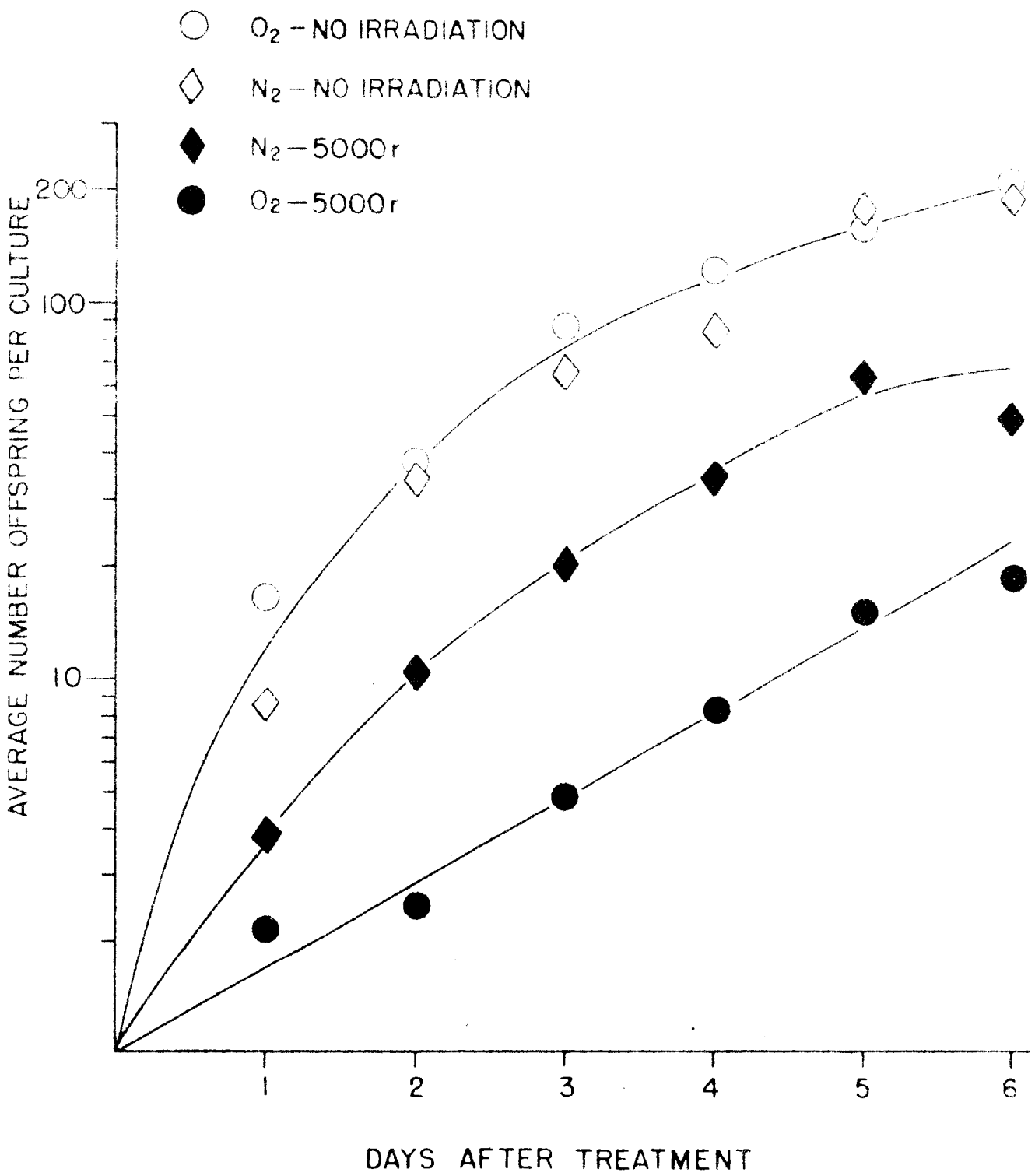

O2 – NO IRRADIATION
N2 – NO IRRADIATION
N2 – 5000r
O2 – 5000r
AVERAGE NUMBER OFFSPRING PER CULTURE
200
100
10
1
2
3
4
5
6
DAYS AFTER TREATMENT

FIG. 2

Figure 3

Relationship between the oxygen concentration at time of irradiation and the viability of the treated males.

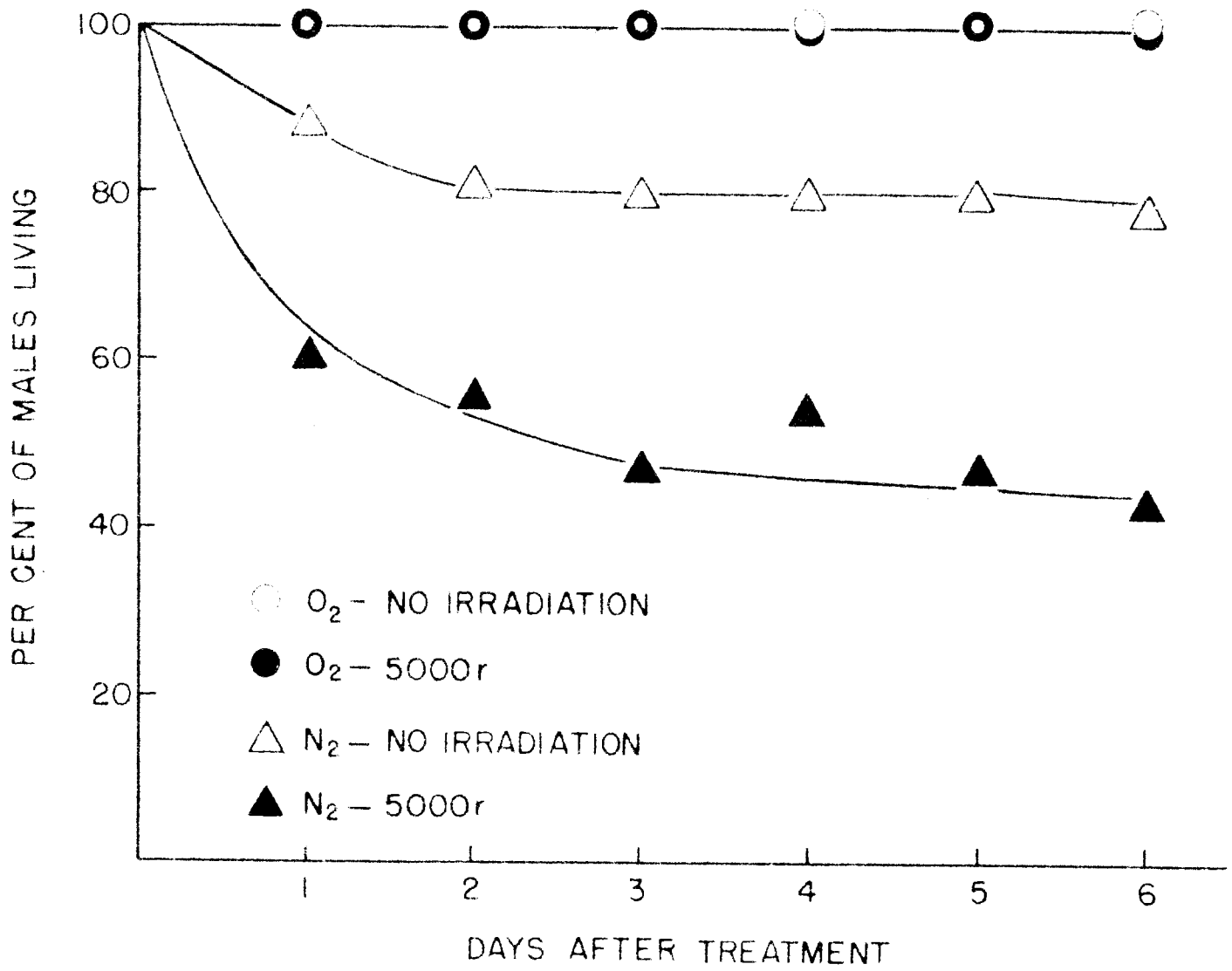

100
80
60
40
20
PER CENT OF MALES LIVING
O_2 - NO IRRADIATION
O_2 - 5000r
N_2 - NO IRRADIATION
N_2 - 5000r
1
2
3
4
5
6
DAYS AFTER TREATMENT

FIG. 3

tension in the sperm is lowered. Since Giles and Riley[3] found that the reduction, as compared to air or oxygen, in the number of induced chromosome aberrations was evident when the Tradescantia inflorescences were irradiated in helium and argon as well as nitrogen, there is no reason to doubt the first assumption. The second assumption seems most likely since the gas transport to the body tissues in small insects with a tracheal respiratory system takes place almost entirely by diffusion[6].

The higher frequency of mutations induced in the flies maintained in oxygen at the low temperature is in agreement with the results expected if oxygen is an agent influencing the radiosensitivity of genes. A lowering of the temperature would affect at least two processes in such a way that the oxygen tension in the cell would be increased: (1) the solubility of oxygen in water is higher at colder temperatures, (2) with the low rate of metabolism associated with the cold temperature, the oxygen gradient across the cell membrane would be less than at the warm temperature. This would, in turn, raise the oxygen concentration within the cell. It should be noted, however, that the rate of diffusion of the oxygen would be lower at the colder temperature.

If the oxygen tension within the cell is a factor in determining the sensitivity of genes to X rays, the question arises as to its mode of action. At the present time any answer to this question is purely speculative. Increased amounts of dissolved oxygen in the cellular fluids being irradiated would form more of the free radicals which act as strong oxidizing agents and which are known to be produced in water by X radiation. Barron, et al.[7], have published evidence which indicates that it is the oxidizing properties of these radicals which cause the X-ray inhibition of the sulfhydryl enzymes. If such enzymes are necessary in the process of reduplication of particular genes, their inhibition could produce a lethal mutation. On the other hand, recent experiments[8] indicate that organic peroxides, which have rather poor oxidizing properties, are effective mutagenic agents. Thus upon irradiation of cells

with high oxygen tensions, the increased amounts of organic peroxides formed would serve to increase the mutation rate. To date, there is no evidence on which the validity of either mode of action can be established.

Summary.—There is a striking reduction in the number of recessive sex-linked lethal mutations induced in *Drosophila melanogaster* males when they are exposed to X rays while in an atmosphere of low oxygen concentration. Although an increased number of mutations were induced in flies irradiated in oxygen at 2° C. over those treated in oxygen at 27° C., this increase is not due to the temperature *per se* but rather it is apparently caused by a higher oxygen tension within the irradiated sperm at the lower temperature. Additional evidence also indicates that fewer dominant lethal mutations and chromosome aberrations are induced in flies maintained in a near oxygen-free atmosphere during irradiation.

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