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*THE BASIS OF THE OXYGEN EFFECT ON X-IRRADIATED DROSOPHILA SPERM**

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Two hypotheses have been proposed to account for the fact that fewer chromosome aberrations are recovered in organisms exposed to ionizing radiations while in low O_2 concentrations as compared to those treated in air or higher concentrations. On the one hand, the data have been interpreted to mean that there is a differential production in the number of primary breaks with high and low O_2 concentrations.[1-3] On the other hand, the conclusion has been reached that the O_2 is affecting differentially the rejoining process such that more reunion of broken ends occurs in low concentrations.[4] Baker and Edington[5] concluded from studies in Drosophila on the production of recessive lethals and translocations in various O_2 concentrations that the data were compatible with either the differential breakage or the differential reunion hypothesis.

In view of the equivocal results with Drosophila, it seemed advisable to design experiments which would distinguish between the two hypotheses. A study of the induction of dominant lethals could provide the necessary facts. There is strong evidence that the dominant lethals induced in Drosophila sperm are, in the main, the result of inviable chromosome rearrangements (for a discussion of this evidence see Muller[6] and Catcheside[7]). On this basis, the theoretical relationship between dominant lethal production and x-ray dosage has been derived by Lea and Catcheside[8] and extended in a more general form by Haldane and Lea.[9] They conclude that the dose relationship of dominant lethals is very well described by a function with the following three parameters: α, the average number of breaks induced/sperm/1000 r which are available for joining at the time of fertilization; q, the probability that a break will rejoin at fertilization (either restitute or join to form a new arrangement); and D, the dose in kr. Lea and Catcheside[8] found that the theoretical curve which best fitted the experimental data of Catcheside and Lea[10] and Demerec and Fano[11] had the parameters $\alpha = 0.75$ and $q = 0.76$. In these experiments the flies were ex-

posed in air. Now if the differential breakage hypothesis were the valid interpretation of the O_2 effect, then irradiations in N_2 should reduce α but not change q. In view of the reported fact that with chromosome aberrations irradiation in N_2 effectively reduces the dose by a factor of 2.5 as compared to treatment in air, α would be reduced to about 0.3 upon treatment in N_2. In figure 1C is shown the theoretical curves (dotted lines) one would expect to obtain according to the breakage hypothesis when exposures are made in air and N_2.

Material and Methods.—The frequency of dominant lethals, for a given dose of radiation, is subject to such variables as age of males at time of treatment, length of time between irradiation and fertilization, strain used, etc. It is necessary, therefore, to describe in some detail the methods used in the experiments being reported. Virgin Oregon-R males of *Drosophila melanogaster*, which had taken 10–11 days to develop from egg to eclosion, were irradiated 3–4 days after eclosion. Immediately after treatment each male was mated to a single virgin Oregon-R female which was from 4 to 6 days old. Twenty-four hours after mating, the male and female in

TABLE 1

DESIGN OF EXPERIMENTS

	AGE OF SPERM (HRS.) FROM		
DESIGNATION	IRRADIATION TO INSEMINATION	INSEMINATION TO FERTILIZATION	IRRADIATION TO FERTILIZATION
Sperm batch Ia	0–24	0–48	24–48
Sperm batch Ib	0–24	24–72	48–72
Sperm batch IIa	24–48	0–48	48–72
Sperm batch IIb	24–48	24–72	72–96

each vial were separated, the male being mated to a fresh female and the original female being placed alone in a vial containing the egg-laying medium. She was allowed to deposit eggs in this vial for 24 hours (eggs from sperm batch Ia) and then she was transferred to a fresh vial for a final 24 hours of egg laying (sperm batch Ib). The second female with which the male was permitted to mate for 24 hours was also allowed to lay eggs (sperm batch II) over a 2-day period under conditions identical to those of the original female. By use of this design it is possible to follow the dominant lethals induced in at least two successive batches of sperm from the same male as well as to follow the age of the sperm from irradiation to fertilization. In table 1 is shown the manner in which this experimental design relates the egg counts to the age of the sperm.

The egg-laying medium was modified slightly from that described by Carpenter[12] and, in addition, powdered charcoal was added to facilitate counting of the eggs. This medium was poured into 35 × 100-mm. glass vials. A drop of streptomycin solution was added to the surface to cut down bacterial contamination. A 25 × 95-mm. empty glass vial, inverted within

the vial containing the egg-laying medium, served to retain the female and allow her to deposit eggs only within a circular area in the center of the medium. Immediately after the female was removed from the medium, a count was made of the total number of eggs deposited. A count was made of both the hatched and unhatched eggs 24–30 hours later. In some experiments an additional count was made 48 hours after removal of the female but this disclosed no additional hatched eggs (temperature 22–27°C.). Vials in which none of the eggs hatched were not included in the data, since at the moderate dosages used and with the fairly large sample of eggs laid by a female over the 2-day period, practically all these cases must have come from females which were not inseminated.

TABLE 2

EFFECT OF OXYGEN CONCENTRATION AND DOSAGE ON THE INDUCTION OF DOMINANT LETHALS

DOSE (r)	GAS	SPERM BATCH I		SPERM BATCH II		SPERM BATCH I HELD 24 HOURS	
		EGGS COUNTED	PER CENT[a] SURVIVAL	EGGS COUNTED	PER CENT[a] SURVIVAL	EGGS COUNTED	PER CENT[a] SURVIVAL
None	Air	21,336	95.5	20,265	95.6	..	..
1000	N_2	1,477	86.6	1,468	89.5	..	..
	5% O_2	2,027	82.8	1,739	79.7	..	..
	Air	2,250	77.0	2,104	81.3	..	..
2000	N_2	2,895	74.4	3,778	74.4	..	..
	5% O_2	3,812	61.1	4,440	68.4	..	..
	Air	4,232	59.6	3,854	66.9	..	..
4000	N_2	3,763	39.8	3,418	41.7	2,329	43.7
	5% O_2	4,408	24.2	4,037	32.4	2,992	31.4
	Air	4,420	24.1	3,972	30.4	2,501	33.4
7000	N_2	1,504	15.8	1,440	15.4	1,454	18.7
	5% O_2	2,392	6.6	2,229	10.9	1,917	11.1
	Air	1,332	5.1	2,158	11.0	2,246	9.7

[a] The percentage survival given has been corrected for the control survival measured at the same time as the irradiated group.

In each experiment four lots of 20–25 males were used; one lot was not irradiated and served as a control, and the other three lots were irradiated simultaneously in an environment of N_2, 5% O_2 (balance N_2), and air respectively. The various gases were passed over the flies for 10 minutes before, and continuously during, irradiation. Gases and flies were kept at a constant temperature (25 ± 0.5°C.) during treatment. Radiation was administered by a Maxitron machine operating at 250 kvp., 30 ma., 2-mm. Al filter, giving an average intensity of 292 r/min. at the target distance of 49 cm. Dosage measurements were made with a Victoreen 100-r thimble chamber placed in the same location as the treated flies.

Results and Interpretation.—The extensive data gathered in this study have been condensed as much as possible for presentation. Statistical analysis revealed no significant difference between the number of eggs surviv-

ing during the first and second day of egg laying in either the eggs fertilized by sperm batch I or by batch II. Therefore in table 2, which is a compilation of the results, the data from Ia and Ib are lumped as well as the data from IIa and IIb. Also the data from the two or three experiments done at a particular dosage are not kept separate. In the first line of the table are the lumped data from the control experiments, each of which was performed simultaneously with an irradiation. Experiments conducted in N_2, 5% O_2, and pure O_2 under exposure conditions identical to the experimental series except for the lack of radiation gave results similar to the control series in air.

One surprising fact becomes immediately apparent upon examination of the data in this table. The percentage survival is higher in eggs fertilized by sperm of batch II than batch I when the sperm was irradiated in an environment of air or 5% O_2. In N_2, there is no difference in survival of eggs receiving sperm from the two batches. It would thus appear unlikely that the explanation of this phenomenon rests on differential sensitivity, at the time of irradiation, of older and younger sperm. However, as a further check on this point, males were irradiated in the fashion just outlined, but after exposure they were separated individually into vials and held for 24 hours before being allowed to mate. After this period, the experiment was conducted in the same manner as the previous experiments. In the last column of table 1 is given the survival of eggs fertilized with sperm treated in this manner. It is obvious that the survival in this group corresponds to the survival in the second sperm batch and thus an explanation of this phenomenon in terms of differential sensitivity is unequivocally ruled out.

Because of the importance of this increased survival, in interpreting the mechanism of the O_2 effect, it was necessary to determine if this apparent difference rests on a firm statistical basis. Previous workers have shown that the frequency of dominant lethals measured is determined by various extrinsic factors mentioned previously. However, it is not only necessary to hold these factors constant from experiment to experiment, but it is also essential to design the experiments so that variations among the individual males treated can also be taken into account. This is made necessary by the observation that there is much more variation in egg hatch among males given identical treatment than can be accounted for by binomial fluctuations. Therefore, in evaluating the difference between sperm batches, it is necessary to perform an analysis of variance in order that this extraneous variation among males may be taken into consideration. The results of the analysis of variance (arc sine transformation was used) are presented in table 3. It is clearly evident, from the figures in the first line of this table, that eggs fertilized by sperm irradiated in air from batch II definitely have a higher survival value than those fertilized by batch I.

Bonnier and Lüning[13] report just the opposite effect; i.e., a decrease in survival with length of time between irradiation and fertilization. However, an examination of their evidence reveals that, although such a decrease may exist, its effect is not demonstrated until at least 4 or 5 days have elapsed between irradiation and fertilization. They did not observe an increased survival between the first and second day, but this is hardly sur-

TABLE 3

ANALYSIS OF VARIANCE OF DATA FROM IRRADIATIONS PERFORMED IN AIR

	1000 r				2000 r			
SOURCE OF VARIATION	DF	MS	F	PROB.	DF	MS	F	PROB.
Sperm I vs. II-treated	1	142.3	4.5	1–5%	1	403.6	9.0	0.1–1%
Sperm I vs. II-controls	1	0.0	0.0	n.s.	1	9.9	0.2	n.s.
Between experiments	1	317.0	11.1	0.1–1%	1	31.7	0.8	n.s.
Controls vs. treated	1	9552.2	334	<0.1%	1	26,468.5	642	<0.1%
Among males[a]	48	28.6			62	41.2		
Males × Sperm I vs. II interaction[b]	48	31.4			62	44.9		
	4000 r				7000 r			
SOURCE OF VARIATION	DF	MS	F	PROB.	DF	MS	F	PROB.
Sperm I vs. II-treated	1	61.6	3.1	5–10%	1	313.3	15.8	<0.1%
Sperm I vs. II-controls	1	19.4	0.5	n.s.	1	177.9	9.0	0.1–1%
Between experiments	2	220.5	3.3	1–5%	1	28.4	1.1	n.s.
Controls vs. treated	1	77,530.4	1172	<0.1%	1	56,774.2	2268	<0.1%
Among males[a]	64	66.1			24	25.0		
Males × Sperm I vs. II interaction[b]	32 C 32 T	38.1[c] 19.9			24	19.8		

[a] These mean squares were used to test significance of variation between experiments and between control vs. treated.

[b] These mean squares were used to test significance of variation between sperm I vs. II in controls and in treated.

[c] Because of the absence of homogeneity among the variances comprising this interaction, the pooled mean squares for controls (C) and treated (T) were used separately in calculating F for sperm I vs. II in controls and in treated, respectively.

prising because of the technique of mass matings used in their experiments. Under such conditions only gross changes would be observable because it is impossible to exclude unhatched eggs laid by virgin females, and because the large extraneous variation among sperm from different males cannot be taken into account.

The analysis in table 3 also brings out two incidental facts of which we were not previously aware. As can be seen in the second line, there is no

significant difference between sperm batches within the controls except those run with the 7000 r series. This highly significant lowering of the hatch count was brought about by one experiment in which the males and females were allowed to mate for 12 hours instead of the usual 24. This lowered hatch rate was due almost entirely to a decrease in the eggs hatching during the second day of egg laying. Presumably a mating period of 12 hours under the existing conditions was not sufficient to insure that all females would receive sufficient sperm to last for 2 days of laying. The other fact, made evident by the analysis (line three), is that the three experiments at 4000 r are not real replications, as is also true of the two experiments conducted at 1000 r. We are aware of no obvious reason for our inability to replicate experiments in these cases.

One other fact should be noted regarding this measurably time-dependent recovery of sperm from the effect of irradiation conducted in air. As can be seen from table 1, if this recovery was dependent on the total time elapsing from irradiation to fertilization, then sperm batch Ib and IIa should give the same percentage survival of eggs. The data clearly indicate that this is not true, since the survival percentages of eggs fertilized by the same sperm batch but laid over a 2-day period are the same. Therefore, it must be concluded that this recovery is predominantly localized in time to the period from irradiation of the sperm to insemination of the female.

It is impossible, as was pointed out, to explain this recovery in terms of differential sensitivity and thus differential chromosome breakage. However, if one thinks of recovery from breakage in terms of joining of broken chromosome ends, then the results form a consistent picture. It is known that in Drosophila the broken ends do not rejoin to form *new* arrangements until the time of fertilization. Presumably a tandem alignment of the chromosomes in the sperm as observed by Cooper[14] is responsible for this behavior. Cooper concludes (personal communication) that there is not yet sufficient cytological evidence to determine whether this orientation is present in only a few or in most sperm. In any case, such an alignment does not preclude the possibility of restitution of certain breaks. In terms of the differential reunion hypothesis of O_2 action, the dominant lethal data can be interpreted to mean that, although the same number of primary breaks are induced in air and in N_2, the breaks induced in N_2 are more likely to rejoin and to rejoin more quickly than those induced in air. Since the only type of rejoining possible in the sperm is restitution (excluding sister-strand fusion if the chromosome is split) the end-result would be fewer breaks available at the time of fertilization to form new arrangements.

In figure 1 is plotted the observed frequency of surviving eggs as functions of dosage, sperm batch, and the three concentrations of O_2 used. The curves shown in graphs A and C are theoretical and are derived from the formulae given by Haldane and Lea,[9] while those in graph B merely con-

nect the observed points. As mentioned earlier, Lea and Catcheside[8] conclude that their data and those of Demerec and Fano best fit the curve with the parameters $\alpha = 0.75$ and $q = 0.76$. As can be seen in figure 1A, this curve provides a reasonable fit to the data of sperm batch II which come from the type of experiment most comparable to the previous work. In sperm batch I treated in air, it is assumed that not all the potential restitution has taken place, and thus the average number of breaks remaining at the time of fertilization is higher. When $\alpha = 1.0$, the theoretical curve gives a good fit to these empirical points. The N_2 data are in good agreement with a value of $\alpha = 0.62$. It is shown strikingly (Fig. 1C), that the

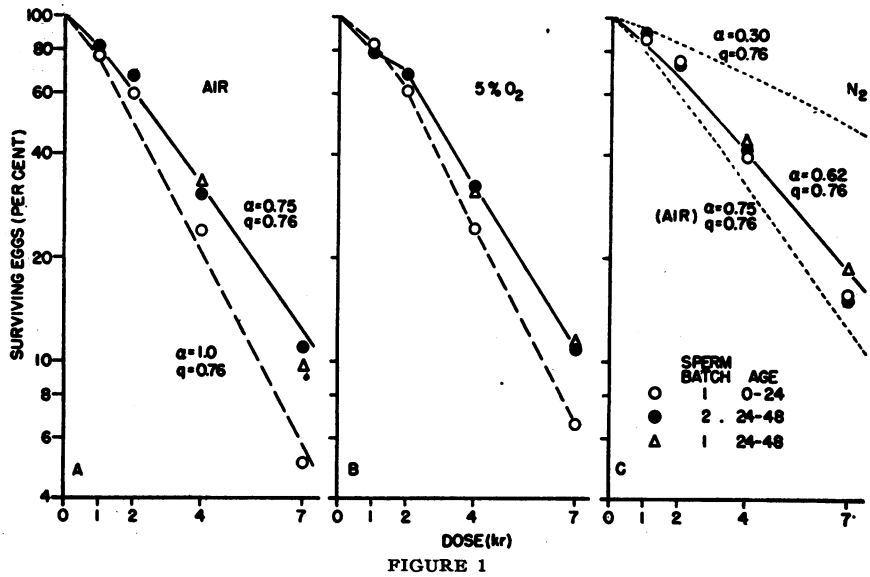

FIGURE 1

Relationship between dosage, oxygen concentration, and sperm batch on the induction of dominant lethals. O = sperm batch I; ● = sperm batch II; Δ = sperm batch I held 24 hours

experimental points fall far short of the values expected were N_2 causing a two and one-half reduction (as compared to air) in the number of breaks remaining at the time of fertilization.[15] These data, however, give no estimate of the actual number of primary breaks produced (assumed to be independent of O_2 concentration), nor do they allow an estimate of the respective probabilities that a broken end will restitute in the sperm when irradiation is carried out in air or in N_2. From dosage experiments in which insemination follows irradiation very closely, some estimate of the value of these sperm parameters might be possible.

In the foregoing interpretation we have assumed that, although the O_2

concentration affects the ability of a broken chromosome end to restitute in the sperm, it does not influence its ability to rejoin at the time of fertilization. This assumption would appear to be necessary in view of the large O_2 effect (over four times as many translocations recovered in air as in N_2) observed with chromosome translocations in Drosophila.[5] The necessity for this assumption can be seen from the following arguments. A reduction in the value of α from 0.75 to 0.62 is not sufficient, according to the calculations of Lea and Catcheside,[8] to account for a fourfold reduction in the number of viable rearrangements. These calculations also show that if the same number of breaks were available at the time of fertilization in N_2-treated and air-treated sperm and if in the former rejoining of broken ends was more likely, then more rearrangements should be recovered in the N_2-treated sperm than the ones exposed in air. Therefore, if the O_2 concentration also affected the rejoining ability at fertilization, a still smaller difference would be expected between the frequency of translocations recovered in air and in N_2. There still remains the problem as to the cause of the large O_2 effect with translocations. It is possible that this discrepancy between the dominant lethal and the translocation data may be caused by the fact that the latter experiments were conducted at 8°C., the former at 25°. Previous work[16] with recessive lethals indicated a larger O_2 effect at the low temperature, but whether or not this is the explanation will have to await further experimentation.

Discussion.—Riley, Giles, and Beatty[3] argue against the reunion hypothesis of O_2 action on the basis of two lines of evidence: (1) Experiments conducted using Tradescantia (Giles and Riley[1]) have shown that changing the O_2 concentration immediately after irradiation, when some of the induced breaks still remain free, has no effect on the yield of chromosome aberrations. (2) The yield of chromatid exchanges varies with intensity of radiation in a similar manner when the irradiation is carried out in N_2 or in O_2.[3] Giles and Riley point out, concerning the first line of evidence, that the experiments do not exclude the possibility that the subsequent behavior of a broken end in rejoining is determined by conditions existing at the time the break is produced. Thus under this possibility, only the O_2 concentration at the time of irradiation would affect the yield of aberrations. The second line of evidence is taken by Riley, *et al.*,[3] to indicate that, "the average restitution time is essentially the same for breaks produced in the presence or in the absence of oxygen." However, strictly speaking, the only conclusion that can be implied from their experiment is that the breaks which are to be used *in exchanges* remain open for approximately the same length of time in O_2 and in N_2. Thus the evidence has no direct bearing on the question as to whether breaks formed in N_2 are more likely to restitute than those induced in O_2 or whether the restitution takes place more quickly. Also it should be noted that while the frequency of chromatid ex-

changes increases with intensity, there is no compensatory decrease in the frequency of chromatid breaks as might be expected if, as is generally assumed, the exchanges arise from two originally produced chromatid breaks. This may mean that there are two types of breaks produced; those which can either restitute or join in a new arrangement, and those which remain permanently broken and subsequently appear as chromatid or isochromatid breaks.

It is generally thought that chromatid deletions and exchanges come from chromatid breaks. However, consideration should be given to the likely possibility that isochromatid breaks can also produce deletions and exchanges when one or more of the broken chromatids restitute. If we keep this possibility in mind, and if we assume that the lack of O_2 makes more likely the restitution of breaks capable of joining, then certain observations of Riley, *et al.*,[3] become more meaningful. A lowering of the O_2 tension during irradiation would markedly decrease the frequency of chromatid exchanges and isochromatid deletions. However, it would be expected that the frequency of chromatid deletions would not be lowered as much since restitution of one of the broken chromatids in an originally produced isochromatid break would increase the number of recovered chromatid deletions. These workers observed a much lower O_2-He dose ratio in the case of chromatid deletions as compared with either exchanges or isochromatid breaks. As they point out, such a difference would not be expected on the basis that O_2 concentration is affecting the initial number of breaks induced.

Support for the reunion hypothesis comes from the following four observations: (1) The very low frequency of endosperm mosaics formed (in contrast with interstitial deletions) in maize when irradiation is carried out in N_2 as compared to air (Schwartz,[4] and unpublished). These mosaics are formed when breaks do not restitute and sister-strand fusion initiates the chromatid type of bridge-breakage-fusion cycle. (2) Dominant lethals in Drosophila are not reduced with irradiation in N_2 to nearly the extent one would expect on the breakage hypothesis. (3) The demonstrated recovery from dominant lethals (interpreted as restitution of some breaks) which is dependent upon the time elasping between irradiation and insemination in sperm treated in air but not demonstrably dependent in N_2-treated sperm. (4) The previously mentioned low dose reduction observed with chromatid deletions in Tradescantia upon exposure in He.

From the available data, there is little reason to believe that the frequency of induced primary breaks is affected by O_2 concentration. Rather, we envisage the substances induced by the interaction of O_2 and x-rays as ones which produce chromosome breaks less capable of subsequent rejoining than the breaks induced in the absence of O_2. In Drosophila, these substances are effective only on the process of restitution, and apparently extend the average length of time between breakage and restitution. It re-

mains to be seen whether the O_2 concentration affects the general rejoining in other organisms in which the process of restitution and rejoining to form new arrangements are not separated in time.

Summary.—A study of the relation between x-ray dosage and O_2 concentration on the induction of dominant lethals in mature sperm of *D. melanogaster* shows that the frequency of dominant lethals induced upon irradiation in N_2 is reduced much less than would be expected on the hypothesis that O_2 concentration is affecting the number of primary breaks induced. Fewer dominant lethals are recovered in sperm exposed in air when at least 24 hours have elapsed between treatment and insemination than when insemination very shortly follows treatment. This effect is not observed in N_2-treated sperm. These data can be interpreted on the basis that the O_2 concentration affects the amount of restitution of chromosome breaks taking place in the sperm. A low O_2 concentration during irradiation makes restitution more likely, and the broken ends apparently restitute more quickly. Therefore, the data lend support to the differential reunion hypothesis of O_2 action rather than to the differential breakage hypothesis.

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