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Juli/August 1964

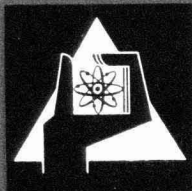
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Evidence for Differential Radiosensitivity rather than
Recovery in Sperm Samples from X-Irradiated
Drosophila Melanogaster Males

The Dose-Dependence of X-Chromosome Loss and Non-
disjunction Induced by X-Rays in Oocytes of
Drosophila Melanogaster

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EVIDENCE FOR DIFFERENTIAL RADIOSENSITIVITY RATHER THAN
RECOVERY IN SPERM SAMPLES FROM X-IRRADIATED
DROSOPHILA MELANOGASTER MALES

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Received March 2, 1964

IT is well established that sperm released the first day after the X-irradiation of 3 to 4 day old *Drosophila melanogaster* males shows a higher mutation frequency than second day sperm (for references see OSTER 1961 and MOSSIGE 1963). Two explanations for this effect have been offered: (1) there is some "recovery" of potential mutational damage during the one day storage period, or (2) the initial radiosensitivity of the sperm sampled during the first day after irradiation is higher than that of the second day. Evidence presented by previous workers as to the likelihood of either explanation has been conflicting (see OSTER 1961 and the discussion following this paper). The experiments reported here support the concept of a lower radiosensitivity of second day sperm rather than that of recovery of damage due to the one day storage period.

MATERIALS AND METHODS

D. melanogaster males, collected during a one day interval and aged 3 to 4 days, were irradiated with 3 kr X rays (150 kv, 6mm aluminum h.v.l., 500 r/min). They were then mated every 24 hours with a large excess of virgin females either immediately (Series 1, consisting of five separate experiments) or after storing them for one day without females (Series 2, consisting of seven separate experiments). In both series, a brood pattern of several one-day broods was obtained. The genotype of the P-males was *B*; that of the P-females γ *sc⁸¹ In49 sc⁸; bw; st* (for explanations of gene symbols see BRIDGES and BREHME 1944). This dual-purpose stock, constructed by OSTER (1958) from similar stocks made by MULLER (1954), allows both recessive sex-linked lethals and 2;3 translocations to be scored from the progeny of the same irradiated males. All flies were cultured at $25 \pm \frac{1}{2}^{\circ}\text{C}$.

RESULTS AND DISCUSSION

The results are presented in Table 1 and in Figure 1. It is evident that the results from the separate experiments of the second day of Series 2, both for lethals and translocations, cannot be combined to a mean value because of the pronounced heterogeneity (the χ^2 -method gives a $P = 0.0009$ for lethals and a $P = 10^{-5}$ for translocations). The results from the other broods in Series 2 and from all broods of Series 1 are homogeneous. For both the lethal and translocation tests, the individual experiments of the second day in Series 2 can be classified into two groups: one which has about the same mutation frequency as the first day of Series 1 (both the translocation and lethal frequencies of Experiments

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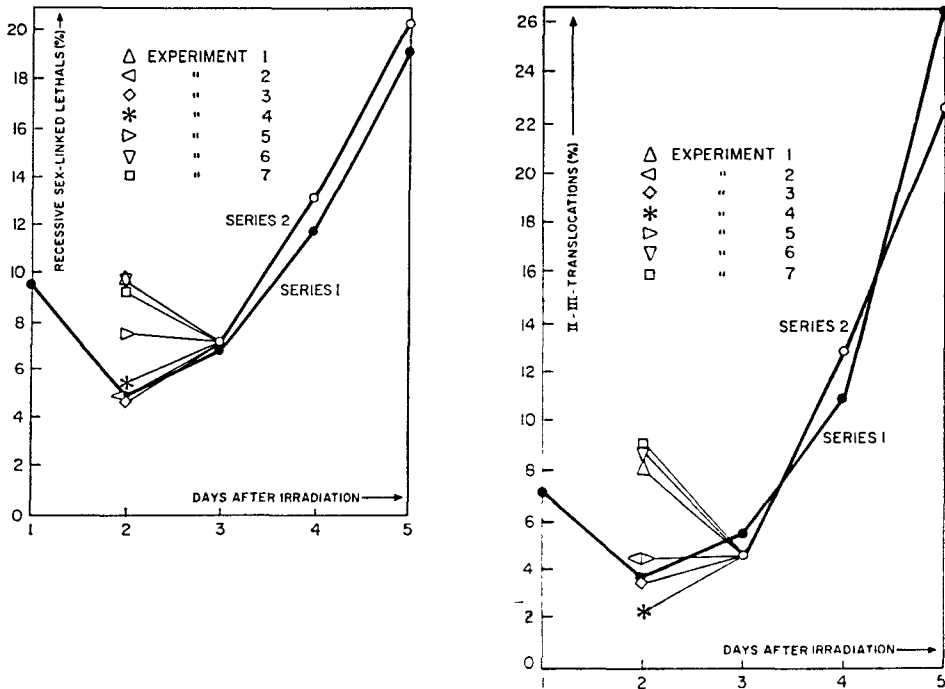


FIGURE 1.—The frequencies of sex-linked lethals and 2;3 translocations from daily broods after irradiating 3 to 4 day old *Drosophila* males with 3000r X rays. The males were mated either immediately after irradiation (Series 1) or after being kept for one day without females (Series 2). For the second day in Series 2 the separate experiments are represented individually (see text for explanation). Frequencies of spontaneous mutations have not been subtracted since they are negligibly low compared to the induced ones. Note that Series 2 starts with Day 2.

1, 6 and 7), and another group which resembles the second day of Series 1 (both the translocation and lethal frequencies of Experiments 2, 3 and 4 and the translocation frequency of Experiment 5). The lethal frequency of Experiment 5 is intermediate. These results are difficult to reconcile with the recovery hypothesis, which would predict a greater consistency between the mutation frequencies of the second day of Series 1 and 2, especially since the experimental conditions were carefully controlled in all of these experiments. It is doubtful that a recovery mechanism is acting in some experiments and not in others. The following explanation, which was suggested by MULLER (1963) when citing our unpublished results together with similar unpublished data by W. E. TRAUT, seems more likely. If we assume that in Series 2, spermatogenesis has proceeded during the one day waiting period, both the typical first and second day sperm are available for fertilization on the second mating-day. Then, experimental factors, which are difficult to control from one experiment to the other, such as the ratio of F_1 flies derived from either Day 1 sperm or Day 2 sperm, could lead to the heterogeneity obtained for Day 2 in Series 2. One wonders, however, why the mutation frequencies of most of the individual experiments of the second day of

TABLE 1

The percent frequencies of sex-linked lethals and 2;3 translocations from Drosophila males irradiated with 3000r X rays

	Day 1	Day 2	Day 3	Day 4	Day 5
<i>Sex-linked lethals</i>					
Series 1*					
Experiments					
1-5	9.6(301/3154)	4.9(131/2682)	6.9(137/1987)	11.8(87/735)	19.2(5/26)
Series 2					
Experiment					
1		9.7(45/466)	13.8(8/58)	18.2(4/22)	50.0(1/2)
2		4.9(25/515)	7.8(30/387)	14.3(18/126)	50.0(4/8)
3		4.7(26/554)	7.6(47/622)	18.2(32/176)	16.7(1/6)
4		5.5(29/530)			
5		7.6(66/864)	5.2(37/706)	12.6(24/190)	25.0(1/4)
6		9.7(9/93)	8.5(28/337)	10.2(22/216)	9.5(2/21)
7		9.3(79/849)	7.6(52/684)	11.8(28/237)	12.5(1/8)
Mean for Series 2†			7.2(202/2794)	13.2(128/967)	20.4(10/49)
<i>2;3 translocations</i>					
Series 1					
Experiments					
1-5	7.2(160/2213)	3.7(80/2195)	5.5(76/1390)	10.9(32/295)	26.7(4/15)
Series 2					
Experiment					
1		8.1(17/209)	20.8(5/24)‡	20.0(2/10)	
2		4.5(16/355)	3.4(7/203)	11.8(6/51)	16.7(1/6)
3		3.5(14/398)	5.5(24/438)	14.3(14/98)	0.0(0/1)
4		2.3(8/349)			
5		4.5(28/621)	3.1(18/588)	12.4(17/137)	
6		8.8(5/57)	5.3(14/262)	12.3(15/122)	27.2(3/11)
7		9.1(67/740)	4.8(22/460)	12.6(12/95)	25.0(1/4)
Mean for Series 2†			4.4(85/1951)	12.9(66/513)	22.7(5/22)

* The males were mated either immediately after irradiation (Series 1) or after being kept for one day without females (Series 2).

† Not calculated for Day 2 because of heterogeneity (see text).

‡ Day 3 of Series 2 (translocations) is homogeneous only after eliminating Experiment 1. The mean therefore has been calculated without this experiment.

Series 2 resemble either the first or the second day of Series 1 instead of being more intermediate. A further factor responsible for the high variability of the results from the second day of Series 2 might be variation in the amount of sperm ejaculated without copulation or in the resorption of unused sperm, as was suggested by LÜNING (1952). The progression of spermatogenesis during the one day waiting period is demonstrated by the fairly good agreement between the mutation frequencies of corresponding broods (see Figure 1), though the later broods are based on only small numbers of chromosomes tested as is typical for brood pattern experiments.

The mutation frequency of sperm X-rayed in the storage organs of *Drosophila* females does not change appreciably within four 4-day periods of egg laying after irradiation as was demonstrated by TRAUT (1962) as well as by OSTER (personal communication). This indicates a lack of recovery in stored mature sperm and further supports the differential sensitivity hypothesis.

Results from experiments by NORDBACK and AUERBACH (1957), performed in a similar way as ours, were tentatively interpreted differently, i.e. by the recovery hypothesis. We feel, however, as did these investigators themselves, that the number of separate experiments in this work and the amount of agreement between them was not large enough to allow this work to be considered as establishing that interpretation.

On the other hand the already mentioned experiments by TROUT (1964) and those of TROSKO (1964) led to the conclusion that differential radiosensitivity rather than recovery due to storing is the explanation for the low mutation frequency of the second day after irradiation. The possibility of sensitivity differences of sperm ejaculated a few days after irradiation had already been discussed by MULLER, HERSKOWITZ, ABRAHAMSON and OSTER (1954).

Causes of the proposed differential sensitivity between first and second day sperm samples are not yet clear. A factor contributing to this effect could be differential oxygenation of the corresponding sperm samples in different sections of the reproductive tract (OSTER 1961).

I wish to express my thanks to Dr. H. J. MULLER, Dr. R. R. RINEHART and Mr. W. E. TROUT for many valuable discussions. The careful technical assistance by Miss U. APITZSCH and Miss I. UFHOLZ is gratefully acknowledged.

SUMMARY

Sperm released the first day after the X-irradiation of 3 to 4 day old males shows a higher mutation frequency than the second day sperm. From a comparison of mutation frequencies of successive one day broods from males which were mated immediately after irradiation with those from males which had been stored for one day before they were mated, it seems likely that the difference between the mutation frequencies of the first and second day is caused by a relatively low initial radiosensitivity of the second day sperm rather than by recovery from radiation damage of the first day sperm owing to the one day storage period.

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THE DOSE-DEPENDENCE OF X-CHROMOSOME LOSS AND NON-
DISJUNCTION INDUCED BY X-RAYS IN OOCYTES
OF *DROSOPHILA MELANOGASTER*

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(Received February 3rd, 1964)

SUMMARY

The dose-dependence of X-chromosome loss and non-disjunction induced by X-rays in oocytes of *Drosophila melanogaster* has been investigated.

The frequency of X-chromosome loss increases faster than linearly with dose, suggesting that this effect is based on both one and two hit events. Whereas the induction of single non-restituting chromosome breaks seems to be a plausible explanation for at least part of the one hit events, the two hit component, though statistically highly significant, is more difficult to interpret. One possibility, the induction of large deletions in the maternal X which would require two breaks and simulate the loss of the whole X-chromosome, was ruled out by special experiments.

The dose-effect relationship for non-disjunction seems too complicated to permit a simple hit explanation. Perhaps radiation-induced stickiness of the chromosome surface, an effect probably not caused by individual hits, prevents the X-chromosomes from separating at meiosis.

Both the X loss and non-disjunction frequency show a "stepwise" increase with X-ray dose. Tentative explanations for this effect are given.

INTRODUCTION

In *Drosophila melanogaster* X-chromosome loss and primary non-disjunction are easily detectable and useful genetical criteria for radiation sensitivity of oocytes. By X loss we mean the formation of eggs without an X-chromosome, regardless of what mechanisms produce this loss, e.g. chromosome breakage or non-disjunction. Primary non-disjunction leads to eggs with two or no X-chromosomes. Both X loss and non-disjunction result in individuals of abnormal sex-chromosome constitution, i.e. XO males and XXY females. The number of XO males is a measure for the X loss frequency, that of XXY females for the frequency of non-disjunction.

After the irradiation of *Drosophila* sperm with 18 MeV electrons the combined frequency of complete loss of either sex-chromosome (X and Y) and partial losses involving the removal of y^+ from the Y-chromosome increases slightly, but significantly, faster than linearly with dose⁶. The dose-exponent of X-ray induced X loss and non-disjunction in *Drosophila* oocytes cannot be determined from the available data on the dose-response relationship of these effects^{4,5}. The present result show

that the frequency of X-ray induced X-chromosome loss increases faster than linearly, suggesting that X-chromosome loss is based on one and two hit events. It is, however, more difficult if not impossible to use the hit concept for interpreting the complicated dose-relationship obtained for induced non-disjunction. A summary of our results has been presented at the Second International Congress of Radiation Research (Harrogate 1962).

MATERIAL AND METHODS

Drosophila melanogaster females of the genotype $y\ sc^{S1}\ In49\ sc^8; bw; st$ were irradiated when 7 ± 1 days old and were mated immediately with normal males (Berlin wild strain). For explanation of the different markers see ref. 3. Actually y is the only marker necessary to distinguish XO males (phenotype y^+) and XXY females (phenotype y) from the regular offspring (y^+ females and y males) in the F_1 . To prove the absence of the Y-chromosome in the y^+ males, these were mated singly with several females and checked for offspring production. Most of them were sterile, and therefore were not the result of secondary non-disjunction, which would produce fertile y^+ males with a Y-chromosome. The P-females were allowed to lay eggs for three days; the F_1 flies arising from eggs laid during this period were scored for X loss and non-disjunction 16 days after the P-females had been irradiated. The temperature was maintained at $25 \pm 1^\circ$ throughout the experiments. The flies were irradiated with 150-kV X-rays at an exposure rate of 500 R/min (current: 15 mA). The h.v.l. was 6 mm Al. Several separate experiments were conducted at each dose to determine the reproducibility of the results. In the lower dose range (0.5–2.5 kR) generally 180 P-females were irradiated per experiment, in the higher dose range (3–5 kR) 360 females were used. Two P-females and two P-males were mated together per vial in the low dose range while 4 females and 4 males were used in the high dose range.

RESULTS AND DISCUSSION

The definition of the X loss and non-disjunction frequency can be based on either the number of regular males (definition 1) or on the number of regular females (definition 2) in the F_1 :

	<i>X loss frequency</i>	<i>Non-disjunction frequency</i>
<i>Definition 1</i>	$\frac{\Sigma XO\ males}{\Sigma XY\ males + \Sigma XO\ males}$	$\frac{\Sigma XXY\ females}{\Sigma XY\ males + \Sigma XXY\ females}$
<i>Definition 2</i>	$\frac{\Sigma XO\ males}{\Sigma XX\ females + \Sigma XO\ males}$	$\frac{\Sigma XXY\ females}{\Sigma XX\ females + \Sigma XXY\ females}$

The results of the separate experiments utilizing the same radiation dose were tested with the χ^2 method for homogeneity. Since no heterogeneity was found these results were combined to an average value for each dose. These average values are presented in Table I and Figs. 1 and 2. Further, we divided the number of degrees of freedom from each set of X loss experiments utilizing the same radiation dose by the corresponding homogeneity- χ^2 (d.f./ χ^2). This procedure, kindly suggested by Dr. A. W. KIMBALL, demonstrates that for either definition of X loss frequency there

TABLE I

THE DOSE-DEPENDENCE OF THE FREQUENCY OF RADIATION INDUCED
X-CHROMOSOME LOSS AND NON-DISJUNCTION

As well the X loss as the non-disjunction frequency can be calculated by referring either to the regular male offspring (definition 1) or to the regular female offspring (definition 2). Note that, as had been demonstrated earlier, both the frequency of induced and spontaneous X loss is higher than that of non-disjunction.

Exposure (kR)	X-chromosome loss (%)		Non-disjunction (%)	
	$\Sigma XO\delta\delta \cdot 100$	$\Sigma XO\delta\delta \cdot 100$	$\Sigma XXY\text{♀♀} \cdot 100$	$\Sigma XXY\text{♀♀} \cdot 100$
	$\frac{\Sigma XY\delta\delta + \Sigma XO\delta\delta}{\text{Definition 1}}$	$\frac{\Sigma XX\text{♀♀} + \Sigma XO\delta\delta}{\text{Definition 2}}$	$\frac{\Sigma XY\delta\delta + \Sigma XXY\text{♀♀}}{\text{Definition 1}}$	$\frac{\Sigma XX\text{♀♀} + \Sigma XXY\text{♀♀}}{\text{Definition 2}}$
0.0	0.13 (9/6700)	0.10 (9/8622)	0.06 (4/6695)	0.05 (4/8617)
0.5	0.45 (50/11082)	0.36 (50/13788)	0.15 (16/11048)	0.12 (16/13754)
1.0	1.16 (101/8724)	1.02 (101/9866)	0.15 (13/8636)	0.13 (13/9778)
1.5	2.20 (150/6805)	1.93 (150/7758)	0.51 (34/6689)	0.45 (34/7642)
2.0	3.34 (134/4009)	2.86 (134/4687)	0.51 (20/3895)	0.44 (20/4573)
2.5	3.81 (171/4491)	3.30 (171/5185)	0.53 (23/4343)	0.46 (23/5037)
3.0	4.98 (382/7676)	4.26 (382/8977)	0.53 (39/7333)	0.45 (39/8634)
3.5	6.69 (510/7623)	5.58 (510/9144)	0.48 (34/7147)	0.39 (34/8668)
4.0	7.31 (318/4352)	6.31 (318/5040)	0.76 (31/4065)	0.65 (31/4753)
4.5	7.87 (263/3342)	6.79 (263/3872)	0.65 (20/3099)	0.55 (20/3629)
5.0	11.36 (350/3082)	9.08 (350/3855)	0.91 (25/2757)	0.71 (25/3530)

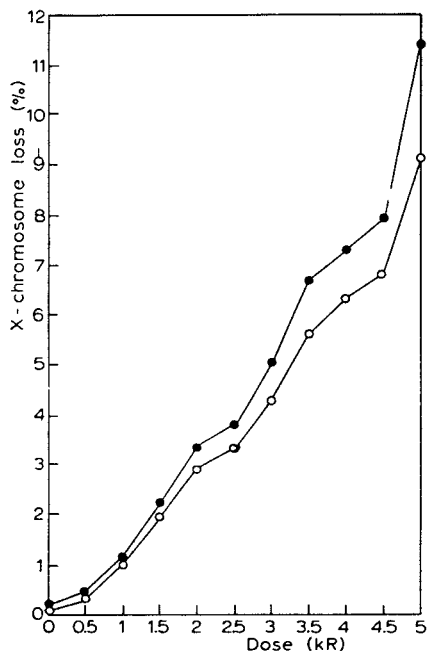


Fig. 1. The dose-dependence of the frequency of radiation induced X-chromosome loss. This frequency can be calculated by referring either to the regular male or the regular female offspring (see text). Thus two curves are obtained. Filled circles represent the results based on male offspring, empty circles those based on female offspring.

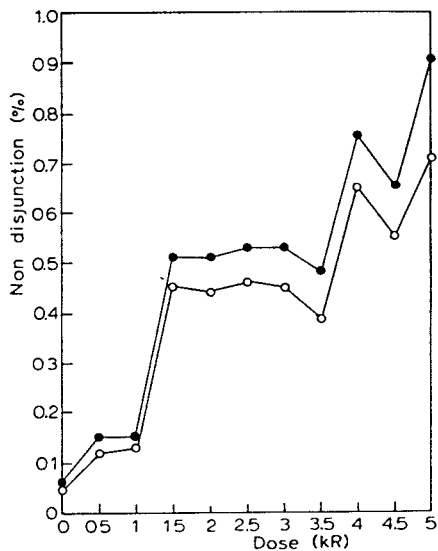


Fig. 2. The dose-dependence of the frequency of radiation induced non-disjunction. This frequency can be calculated by referring either to the regular male or the regular female offspring (see text). Thus two curves are obtained. Filled circles represent the results based on male offspring, empty circles those based on female offspring.

are about as many d.f./ χ^2 -ratios above one as there are below one as would be expected if the data were truly binomial, indicating that our experiments were very well controlled.

The statistical test applied to our dose-response curves is an analysis of variance performed in the following way: If one fits the experimental points of a dose-response curve by a regression polynomial of n^{th} degree when there are $(n + 1)$ doses, one can demonstrate the significance of the terms of the polynomial (first term, second term, ... n^{th} term) by comparing the variation due to a particular term of the regression polynomial with the residual variation. A detailed description of this procedure is found in ref. 11. In these calculations the X loss and non-disjunction frequencies were weighted according to the absolute numbers of gametes tested as outlined in ref. 1.

The regression analysis demonstrates that for either definition of X loss frequency a positive linear as well as a positive quadratic term of the regression are significant ($P < 0.001$). One may therefore conclude that both one and two hit events contribute to the total frequency of induced X loss. As a possible result of one hit events, single chromosome breaks could lead via sister union and anaphase-II-bridges to the loss of the X^s.¹⁴ As suggested by Dr. D. L. LINDSLEY and Dr. D. R. PARKER (personal communication), the two hit component might, at least partly, result from the induction of large deletions in the maternal X, requiring two breaks and leaving only the proximal and distal chromosome ends. With our scheme we would not have been able to distinguish such deleted-X males from XO males. However, this distinction is possible with the following modified technique, suggested by Dr. D. L. LINDSLEY.

We irradiated normal females (strain Berlin wild) with 3500 R, a dose which should lead to a high two hit component of the total X loss frequency, and mated them with $Y^s \cdot X \cdot Y^L$, $In(1)EN$, $In(19)$, $y v f car/O$ males, *i.e.* males carrying an attached X · Y chromosome. The experimental conditions were the same as those in the preceding experiments. In the F_1 , one can distinguish XO males (phenotype $y v f car$) from deleted-X males (phenotype $v f car$, the y being covered by the y^+ of the deleted X). Having an attached X · Y chromosome, the deleted-X males were fertile and their deletions could be verified by appropriate crosses. We obtained a total of 76 XO males compared to only 2 deleted-X males. Most of the 76 XO males are, as can be calculated from our regression, produced by more than one hit. Some of these experiments were performed in Karlsruhe (Germany), leading to 36 XO males and 1 deleted-X male; some in Philadelphia (U.S.A.) in the laboratory of Dr. I. I. OSTER*, where—in good agreement with the former results—the ratio was 40:1. This demonstrates that only a small part of the two hit component is based on large deletions, leaving the question open as to what else this component may be attributed. Nevertheless the statistical analysis of our dose-response curve for X loss strongly suggests the existence of a two hit component in the total X loss frequency.

Fig. 1 shows that there are two “steps” (at 2 and 3.5 kR) in the dose-effect curve for X loss. It is, however, difficult to treat these “steps” statistically. They are possibly correlated with the significant negative 4th and the significant positive 6th and 7th term of the regression. Experiments on sex-linked lethals¹³ and the non-disjunction data presented in Fig. 2 provide further evidence for “step” formation. For a

* These experiments were done under U.S. Atomic Energy Commission Grant AT (30-1) - 2618 to Dr. I. I. OSTER and co-workers.

tentative explanation we assume (1) that our oocyte sample is to a certain degree heterogeneous as regards radiosensitivity, (2) that those oocytes especially sensitive to the induction of X loss are also especially susceptible to the induction of dominant lethals. Then the dose-effect curve is expected to flatten with increasing dose since the more sensitive oocytes might be eliminated via production of dominant lethals. A flattening of the dose-response curve for these reasons has been clearly demonstrated in experiments with sex-linked lethals^{9,10,12}. The X loss curve, now based on more resistant oocytes since a relatively high sensitive oocyte fraction has been eliminated, might rise again with further increasing dose. This flattening and rising again of the X loss curve would give the impression of a "step". The elimination of another sensitive oocyte fraction could result in a second "step" so that two "steps", one at 2 kR the other at 3.5 kR, could be produced.

It is more difficult to interpret our dose-effect results on non-disjunction (3rd and 4th column of Table I and Fig. 2). The statistical analysis for either definition of the non-disjunction frequency (see above) gives a significant positive linear term ($P < 0.001$) as well as a significant negative 7th term ($P < 0.05$) and a significant positive 9th term ($P < 0.05$). These higher terms are possibly correlated with the stepwise increase of non-disjunction frequency with dose (see Fig. 2), which may be principally explained in the same way as in the case of our X loss results. However, it is problematic to correlate the linear term with a one hit mechanism as it is difficult to imagine such a mechanism which would produce a chromosomal change preventing the two X-chromosomes from disjoining. Perhaps, radiation induced non-disjunction is not based on "hit events" at all. It is known that radiation can produce a reversible stickiness of the chromosomal surface^{2,7}. This effect, which is probably not caused by individual hits, could prevent the X-chromosomes from separating at meiosis and might be independent of radiation dose at certain dose ranges. This could explain the independence of non-disjunction frequency on dose noted in the dose range from 1.5 to 3.5 kR (see Fig. 2). Then, an alternative explanation for the "steps" of the dose-effect curve for non-disjunction would be available. Those parts of the curve parallel to the abscissa (see Fig. 2) would not be the result of the selective elimination of the more sensitive oocytes from the oocyte sample but could be considered as an expression of the independence of non-disjunction frequency on radiation dose at these dose ranges.

ACKNOWLEDGMENTS

I am indebted to Dr. H. J. MULLER, Dr. I. I. OSTER and Dr. R. R. RINEHART for valuable discussions as well to Dr. I. I. OSTER and Dr. D. L. LINDSLEY for providing me with some of the *Drosophila* stocks used in these experiments. Many thanks are due to the careful technical assistance by Miss U. APITZSCH, Miss I. UFHOLZ and Mrs. A. TRAUT. I am thankful to Dr. W. HÄFELE and his group of mathematicians for their assistance in the statistical calculations, performed with the computer.

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