GENETIC EFFECTS OF LOW X-RAY DOSES IN DROSOPHILA

Progress Report

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Summary

We have continued our investigation of the relationship of X-ray dose to frequency of new sex-linked lethals for oögonia of *Drosophila* treated with low X-ray doses. Our purpose is to determine whether sex-linked lethal frequencies induced by doses of 20, 100, 200 and 500 R in immature, metabolizing germ cells are close to the expected values obtained by extrapolation from high dose results on a linear model.

At this date our results seem to indicate that this is not the case. However, more data are needed to substantiate these findings, especially for the 100, 200 and 500 R dose points. Since our last report over 100,000 more X-chromosomes were treated and analyzed, each from a different P_1 -female.

Our results to date, from over 323,700 X-chromosomes processed, gave the following new lethal frequencies with standard errors (uncorrected for controls):

0R (control), $.141 \pm .013\%$ (87,529 tests); 20R, $.153 \pm .015\%$ (66,686); 100R, $.131 \pm .014\%$ (64,364); 200R, $.174 \pm .025\%$ (28,241); 500R, $.288 \pm .022\%$ (60,692); 3000R, $1.438 \pm .127\%$ (8,830); 6000R, $2.829 \pm .193\%$ (7,387).

At this time, only the results from 500R differ significantly from the control rate. However, the induced component is less than expected from linear extrapolation based on the results from 3000 and 6000R.

I. PURPOSE OF THE INVESTIGATION

The purpose of our large-scale investigation is to determine the quantitative relationship of X-ray dose to induced mutation frequency in immature germ cells of *Drosophila*, for low doses up to 500 R.

Linear proportionality has been firmly established for mature germ cells (spermatozoa) from very high doses down to only 8 R; likewise, the same principle applies for immature germ cells (gonia) when intermediate to high doses are tested. However, this might not be the case for gonial cells irradiated with very low doses.

Oftedal (1964) irradiated spermatogonia in late embryonic stages with low X-ray doses. He found that, for doses up to 155 R, the per roentgen yield of mutations was much higher than expected on a linear model when extrapolating from results obtained with high doses. The reverse was found for 300 R; here, the per roentgen yield was less than predicted.

To confirm and expand these findings would be of great importance for the estimate of radiation hazards, especially since immature germ cells persist for a long time during the life of an organism. Therefore we decided on a parallel and complementary experiment, in which adult females would be irradiated with low doses, and their offspring from germ cells in oogonial stage at treatment time analyzed for newly arisen sex-linked lethals.

II. EXPERIMENTAL PROCEDURES

No changes have been made in experimental procedures during this grant period. Methods of irradiation, and the breeding analysis to detect newly arisen sex-linked lethals have been described in detail in previous progress reports. A short outline and diagram of the breeding scheme will again be included in this report.

Young adult females are irradiated with doses of 0 R (control), 20, 100, 200, and 500 R. Occasionally, high doses of 3000 and 6000 R were given to check whether mutation frequencies are in reasonable agreement with values given in the literature for high doses. All irradiations are performed at 250 Kvp, at a rate of 75-85 R min., from a G.E. Maxitron-300 X-ray machine.

By treating females instead of males, we avoid the loss of those X-chromosome mutations which are subject to germinal selection. For this reason, mutation rates for X-chromosomes irradiated in diploid females give a more accurate estimate than from irradiated hemizygous males.

The treated P_1 -females are mated, brooded repeatedly with an excess of males until day 13 after irradiation; then, they are cultured individually without males. Careful inspection of the progeny (F_1) is a standard procedure to eliminate cultures of P_1 -females which were heterozygous for a pre-existing lethal.

By testing only one single daughter (F_1) from each experimental P_1 -female, clusters of mutations of identical origin are avoided. The F_2 -generation is inspected for new sex-linked lethals, and

very strict numerical standards are used for retesting and classification of all suspected lethals.

Various investigators have used different criteria for lethality. In some cases, only "complete" lethals are counted, in others, also partial lethals. In these investigations we count as lethal mutations all cases where the ratio of males representing the experimental chromosome (y dow) to heterozygous sib-females is less than, or equal to 5% of expectation. This "expectation" is empirically determined from F_2 -progeny counts of non-irradiated control cultures which show obvious good viability of the y dow males.

Various other precautions include: Coding of all dose groups; rotation in the handling of the various treatment groups among the different members of our research group. Periodically, about every 6 months, the stock which supplies the P_1 -females for the experiments is freed from lethals, visible mutations, and more severe detrimental.

III. RESULTS TO DATE

Our last progress report was based on data from 130 experiments, with over 220,000 X-chromosomes tested. We have now completed 175 experiments, with over 323,700 X-chromosomes successfully analyzed for lethality, each from a different treated or control P_1 -female.

Experiments 1-33 included only doses of 0, 20, and 500 R; from experiment 34 on a 100 R dose was added, and the 200 R dose was initiated only with experiment 121 (January, 1972). All

these doses are given in every experiment, with rare exceptions. However, we have reduced the number of tests for 20 R during the past year, and are in the process of phasing out this dose, since its mutation frequency is so close to the control rate that it would take an enormous amount of effort and expense to try to determine the induced component.

The results for low dose tests for experiments 1-175 are summarized in Tables 1 through 3. Table 1 subdivides the data for sex-linked lethal frequencies into 8 groups of consecutive experiments with approximately 10,000 tests in the largest treatment groups. The grand totals and mean mutation frequencies are given on the bottom line. This table is intended to show the amount of variation of the different treatment groups as the experiment progressed. The control rates appear to be lower in later experiments. Frequencies for the 20 R and 100 R groups are not significantly different from the control rates, whereas the 500 R dose shows a significant difference. Too few data have been collected as yet in the 200 R groups to make a judgement. The 100 R group has been rather exceptional in that its lethal frequencies have been more often below the control rate than above it (in 6 out of the 8 groups listed in Table 1). However this difference, though intriguing, is not statistically significant at this time.

In Table 2 cumulative frequencies for new sex-linked lethals are given with standard errors, and are arranged in the same manner as in Table 1. As more data accumulate, mutations frequencies tend to stabilize.

In Table 3 the data are subdivided into only 4 consecutive groups of experiments, with about 20,000 chromosomes tested in the main dose groups. The mean mutation rates, based on the total data, are listed on the bottom line. It appears from this table that the spontaneous mutation frequencies do not show a random variation, but have become considerably lower during the second half of our investigations, possibly because we selected for a more vigorous stock to furnish the parent females. However, this may simply be due to chance variation.

Last year we were fortunate to have the assistance of a biostatistician, Dr. Roy C. Milton, in analyzing our data and the setting up of several computer programs.

In one of the programs the confidence intervals for the observed lethal frequencies (p) are computed. The upper and lower limits are obtained on the assumption of linearity of dose and lethal frequency. For the data from experiments 1-175 we obtained these confidence limits:

PERCENT MUTATION FREQUENCY WITH CONFIDENCE
INTERVAL BASED ON BINOMIAL DISTRIBUTION

Dose	Leth. Freq., $p(\%)$	S.E. (p)	95% Confidence Interval
0 R	.1405	.0127	(.1170, .1679)
20 R	.1530	.0151	(.1249, .1859)
100 R	.1305	.0142	(.1042, .1618)
200 R	.1735	.0248	(.1286, .2297)
500 R	.2883	.0218	(.2480, .3353)
1000 R	1.4383	.1267	(1.2179, 1.7338)
6000 R	2.8293	.1929	(2.5350, 3.3272)

It can be readily seen that the mutation frequencies from 0 R through 200 R all overlap. In the low dose range, only the frequency for 500 R is significantly different from the control rate, though its induced component is only .147%. 500 R seems to be the so-called "doubling dose" for our material.

Our high dose treatments with 3000 and 6000 R give us a value of .44% sex-linked lethals induced for each 1000 R; this is within the range expected from previous high dose work with gonial cells, approximately .50% per 1000 R. The slight difference might be due to our more stringent criteria for defining lethality.

Therefore we find that a dose of 500 R seems to induce only 66.8% of the frequency of sex-linked lethals expected by extrapolating from our own high dose results on the assumption of linearity. This may indicate that there indeed is a 2 track-2 hit component to the induced mutations at the high doses, even though they appear to increase proportionally to the dose.

RECENT PUBLICATION

Meyer, Helen U. and Seymour Abrahamson 1973 Dose kinetics for sex-linked lethal rates induced in oogonia of *Drosophila melanogaster* by low X-ray doses. (Abstr.) Proc. Internatl. Congr. of Genetics (Berkeley, Calif., August 1973) Genetics, 74 (supplement) s181.

X-RAY DOSES

	0R (Control)	20R	100R	200R	500R
Exp. No.	No. lethals/ No. tests	No. lethals/ No. tests	No. lethals/ No. tests	No. lethals/ No. tests	No. lethals/ No. tests
1-30	20/10,252 (.195%)	15/9,568 (.157%)	--	--	9/3,241 (.278%)
31-48	31/10,053 (.308%)	17/9,778 (.174%)	11/5,502 (.200%)	--	10/4,678 (.214%)
49-71	17/11,124 (.153%)	19/10,551 (.180%)	10/7,695 (.130%)	--	30/6,654 (.451%)
72-90	15/10,701 (.140%)	16/10,929 (.147%)	11/9,276 (.119%)	--	17/8,003 (.212%)
91-110	8/11,268 (.071%)	21/11,055 (.190%)	13/10,170 (.128%)	--	17/8,107 (.210%)
111-130	12/11,382 (.105%)	6/6,893 (.087%)	8/10,451 (.076%)	15/6,414 (.234%)	29/9,883 (.293%)
131-152	13/11,511 (.113%)	4/5,356 (.075%)	12/10,839 (.111%)	10/11,233 (.089%)	35/10,020 (.349%)
153-175	7/11,230 (.062%)	4/2,556 (.157%)	19/10,431 (.182%)	24/10,594 (.227%)	28/10,106 (.277%)
1-175	123/87,529 (.141%)	102/66,686 (.153%)	84/64,364 (.131%)	49/28,241 (.174%)	175/60,692 (.288%)

Table 1: Results of sex-linked lethal tests, subdivided into 8 groups of consecutive experiments with approximately 10,000 tests in the main treatment groups.

X-RAY DOSES

Exp. No.	0R	20R	100R	200R	500R
	% l. ± s.e.	% l. ± s.e.	% l. ± s.e.	% l. ± s.e.	% l. ± s.e.
1-30	.195 ± .044 (10,252)	.157 ± .040 (9,968)	--	--	.278 ± .092 (3,241)
1-48	.251 ± .035 (20,305)	.165 ± .029 (19,346)	.200 ± .060 (5,502)	--	.240 ± .055 (7,919)
1-71	.216 ± .026 (31,429)	.171 ± .024 (29,897)	.159 ± .035 (13,197)	--	.336 ± .048 (14,573)
1-90	.197 ± .022 (42,130)	.164 ± .020 (40,826)	.142 ± .025 (22,473)	--	.292 ± .036 (22,576)
1-110	.170 ± .018 (53,398)	.170 ± .018 (51,881)	.138 ± .020 (32,643)	--	.270 ± .030 (30,683)
1-130	.159 ± .016 (64,780)	.160 ± .016 (58,774)	.123 ± .017 (43,094)	.234 ± .060 (6,414)	.276 ± .026 (40,566)
1-152	.152 ± .014 (76,299)	.153 ± .015 (64,130)	.121 ± .015 (53,933)	.142 ± .028 (17,647)	.291 ± .024 (50,586)
1-175	.141 ± .013 (87,529)	.153 ± .015 (66,686)	.131 ± .014 (64,364)	.174 ± .025 (28,241)	.288 ± .022 (60,692)

Table 2: Cumulative frequencies of new sex-linked lethals, with standard errors, for 8 groups of consecutive experiments. Number of X-chromosomes tested in parentheses.

DOSE:	<u>0 R</u>	<u>20 R</u>	<u>100 R</u>	<u>200 R</u>	<u>500 R</u>
	% l. $\pm$ s.e.	% l. $\pm$ s.e.	% l. $\pm$ s.e.	% l. $\pm$ s.e.	% l. $\pm$ s.e.
Exp. 1-48	.251 $\pm$.035 (20,305)	.165 $\pm$.029 (19,346)	.200 $\pm$.060 (5,502)	--	.240 $\pm$.055 (7,919)
Exp. 49-90	.147 $\pm$.026 (21,825)	.163 $\pm$.028 (21,480)	.124 $\pm$.027 (16,971)	--	.321 $\pm$.047 (14,657)
Exp. 91-130	.088 $\pm$.020 (22,650)	.150 $\pm$.029 (17,948)	.102 $\pm$.022 (20,621)	.234 $\pm$.060 (6,414)	.256 $\pm$.038 (17,990)
Exp. 131-175	.088 $\pm$.020 (22,749)	.101 $\pm$.036 (7,912)	.146 $\pm$.026 (21,270)	.156 $\pm$.027 (21,827)	.313 $\pm$.039 (20,126)
Exp. 1-175	.141 $\pm$.013 (87,529)	.153 $\pm$.015 (66,686)	.131 $\pm$.014 (64,364)	.174 $\pm$.025 (28,241)	.288 $\pm$.022 (60,692)

Table 3: Frequencies of new sex-linked lethals, with standard errors, from X-rayed oogonia (uncorrected for control). Total number of chromosomes tested in parentheses.

BREEDING SCHEME

P₀: Balanced stock furnishing virgin females:

$$\begin{array}{c} \text{♀♀} \quad \frac{y \quad \text{dow}^*}{\text{Df}(1^{J1} \quad y \quad \text{ac}) \quad \text{sc}^{S1} \quad B \quad \text{InS} \quad w^a \quad \text{sc}^8} \quad \times \quad \frac{\text{Df, Basc}}{y+\text{sc}^8.Y} \quad \text{♂♂} \end{array}$$

P₁:

$$\begin{array}{c} \text{Virgin} \quad \frac{Y \quad \text{dow}^{**}}{\text{Df, Basc}^-} \quad \text{♀♀} \quad \times \quad \frac{y^{3ld} \quad w^{FM6} \quad B, \quad \text{InsFM6}}{Y^+ \quad (\text{normal } Y)} \quad \text{♂♂} \end{array}$$

P₁ females X-rayed, mated en masse in bottles and transferred every 3 or 4 days to fresh culture medium until day 13 after irradiation.

Surviving fertilized females bred individually in vial cultures. No males present.

F₁: To exclude preexisting lethals, each culture is inspected for presence of a reasonable proportion of y dow males; if satisfactory:

$$\begin{array}{c} \text{One single} \quad \frac{Y \quad \text{dow}}{y^{3ld} \quad w^{FM6} \quad B, \quad FM6} \quad \text{F}_1 \text{ daughter} \quad \times \quad \frac{\text{"automatically virgin" (*)}}{Y^+} \quad \text{♂♂} \end{array}$$

Cultures raised at 25°C

F₂: Scored for y dow males; absence or scarcity indicates viability mutation (lethal or partial lethal).

F₃: Confirmation cross.

(*) Note: dow males are sterile. "Df,Basc" is lethal in combination with a normal Y (Y+), but not with the y⁺ sc⁸.Y which compensates for the lethal effect of the deficiency.

(**) Broken lines indicate irradiated X-chromosomes.