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MASTER

GENETIC EFFECTS OF LOW X-RAY DOSES IN DROSOPHILA

Progress Report

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SUMMARY

We have continued our large-scale experiments concerning the numerical relationship of dose and mutability of immature germ cells (oogonia) at very low X-ray doses.

Young adult females (P_1) receive 0, 20, 100, 200, and 500 R of 250 kv X-rays; the 200 R point was added only recently. Sporadically high doses of 6000 and 3000 R are given to check the potential mutability of our particular X-chromosome (y dow).

P_1 -females are mated and brooded intensively for 13 days. Progeny from cells in oogonial stage at irradiation time are analyzed for new sex-linked lethals. By testing only one single daughter from each treated P_1 -female, clusters of mutations of identical origin are avoided. P_1 -females heterozygous for a pre-existing lethal are excluded.

So far, over 220,000 X-chromosomes have been analyzed successfully in 130 experiments. The following lethal frequencies with standard errors were obtained (not corrected for controls):
0R (control, $.159 \pm .016\%$ (64,780 tests); 20R, $.160 \pm .016\%$ (58,778); 100R, $.123 \pm .017\%$ (43,094); 200R, $.234 \pm .060\%$ (6,414); 500R, $.276 \pm .026\%$ (40,566); 3000R, $1.341 \pm .213\%$ (2,908); 6000R, $2.884 \pm .234\%$ (5,097). Lethal frequencies for 20 and 100 R overlap the control value. A significant difference is shown for 500R, but the induced portion is much less than expected by linear extrapolation from data for gonads irradiated with high doses.

I. PURPOSE OF THIS INVESTIGATION

We have continued experiments investigating the relationship of X-ray dose and induced mutation frequency in immature germ cells, for very low X-ray doses. Since immature germ cells in *Drosophila* have very low frequencies of induced mutations even at high doses, any investigation of low dose effects in such cells is a very large scale undertaking.

Linear relationship of dose and mutagenicity was found for high and intermediate doses when gonial cells are irradiated. The only information on low dose kinetics for immature germ cell stages comes from Oftedal's work (1964), in which spermatogonia in a late embryonic stage of *Drosophila* were irradiated. His results indicate for doses below 300R a per roentgen yield of mutations higher than expected on a linear model from data obtained with higher doses. The reverse was found for the 300 R group; there, the per roentgen yield was less than predicted on the assumption of linearity.

We decided to investigate this question by irradiating adult females and analyzing offspring from germ cells in oogonial stage at time of treatment for newly arisen mutations.

II. EXPERIMENTAL PROCEDURES

Our experimental procedures have been described in great detail in last year's comprehensive report. A short outline of the breeding procedures will be included again with this report.

Young adult females are irradiated with various low doses of 250 kv X-rays. Mutagenic effects of the X-rays are measured by testing their offspring for newly-arisen sex-linked lethal mutations. These P_1 females are then mated, and brooded intensively, with several changes to fresh culture media. Eggs which were mature, or near-mature at time of irradiation are deposited first and used up; later laid eggs will be derived from cells which were in oogonial stage at time of treatment.

By treating females instead of males, we avoid the loss of those X-chromosome mutations which would act as cell-lethals and be subject to germinal selection. For this reason, mutation frequencies for X-chromosomes from irradiated females give a more accurate estimate than from irradiated males.

Since only one single daughter (F_1) is tested from each P_1 -female, possible clusters of mutations of identical origin are avoided. Rigid standards are employed for the elimination of preexisting lethals, and later on for retesting and classification of all new lethals.

The low doses tested in our experiments at the present time are 0, 20, 100, 200, and 500 R. Occasionally, high doses of 3000 and 6000 R are included to check the mutagenic potential of our particular experimental X-chromosome (y dow). The 200 R dose was initiated only at the beginning of this year; this dose was

substituted for the originally proposed (in our last renewal request) dose of 300 R. The 200 R dose was preferred because it was closer to 100 R, where we had noted an apparent depression of mutation frequency below that of the control rate; therefore it seemed more interesting to study 200 R instead of the 300 R dose point. Both the 200 R and the 3000 R dose treatments were only initiated during this present grant period, and therefore relatively few data have yet been accumulated.

Lately, we decreased the number of tests for the 20 R dose because its mutation frequency is almost identical to the control rate, and an immense amount of data would be necessary to detect any induced difference. We now concentrate our efforts on the range 100 R to 500 R.

At this time we have completed 130 experiments. In each experiment, all the doses under investigation at that time were included until very recently; but now a 20 R treatment is sometimes omitted. At first we tested only 0, 20, and 500 R; starting with experiment 34, the 100 R dose was introduced, and the 200 R treatment was added only recently, starting with experiment 111.

III. RESULTS

In this investigation, mutagenic effectiveness of the various X-ray doses is measured by the frequencies of newly arisen sex-linked 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 X-chromosome to heterozygous sib females (in the F_2 generation) is less than 5% of expectation. We determine this "expectation" empirically from F_2 progeny counts of non-irradiated control cultures with obvious good viability of these males.

The results for low dose tests for experiments 1-130 are summarized in Tables 1 and 2. In Table 1 the data for sex-linked lethal frequencies are subdivided into 6 groups of consecutive experiments with approximately 10,000 tests in the largest treatment groups. The grand totals and the mean mutation frequencies are given on the bottom line. This table shows the amount of variation for the various treatment groups as the experiment progressed. The control rates appear to be lower in later experiments. Frequencies for the 20 R group have remained relatively stable in spite of the fluctuations of the corresponding control rates. The same holds for the data of the 500 R group. The 100 R group has been rather exceptional in that its lethal frequencies have been more often below the control rate than above it. However, this difference 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, mutation frequencies tend to stabilize. When all the data are summarized, the rates for 0 and 20 R are almost identical; for 100 R it is somewhat below the control rate, but the standard errors overlap.

X-RAY DOSES

Exp. No.	0R (Control) No. lethals/ No. tests	20R No. lethals/ No. tests	100R No. lethals/ No. tests	200R No. lethals/ No. tests	500R No. lethals/ No. tests
1-30	20/10,252 (.195%)	15/9568 (.157%)	--	--	9/3241 (.278%)
31-48	31/10,053 (.308%)	17/9778 (.174%)	11/5502 (.200%)	--	10/4678 (.214%)
49-71	17/11,124 (.153%)	19/10,551 (.180%)	10/7695 (.130%)	--	30/6654 (.451%)
72-90	15/10,701 (.140%)	16/10,929 (.147%)	11/9276 (.119%)	--	17/8003 (.212%)
91-110	8/11,268 (.071%)	21/11,055 (.190%)	13/10,170 (.128%)	--	17/8107 (.210%)
111-130	12/11,382 (.105%)	6/6893 (.087%)	8/10,451 (.076%)	15/6414 (.234%)	29/9883 (.293%)
TOTALS 1-130	103/64,780 (.159%)	94/58,774 (.160%)	53/43,094 (.123%)	15/6414 (.234%)	112/40,566 (.276%)

Table 1: Results of sex-linked lethal tests, subdivided into 6 groups of consecutive experiments with approximately 10,000 tests in the largest treatment groups.

X-RAY DOSES

	0R	20R	100R	200R	500R
Exp. No.	%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)

Table 2: Cumulative frequencies of new sex-linked lethals, with standard errors, for the same groups of consecutive experiments as in Table 1. Total number of X-chromosomes tested in parentheses.

Mutation frequency for 500 R is significantly above the control value, though its induced component is very low, roughly only .117.

We were fortunate to have the assistance of a biostatistician, Dr. Roy C. Milton, of the Department of Statistics and Computer Sciences, for analyzing our data and setting up several computer programs for us. His services were paid for by the Graduate School.

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 1-130 we obtained these confidence limits:

DOSE	p	<u>CONFIDENCE INTERVALS FOR p</u>	
		95%	98.726%
0 R	.001590	(.001298, .001928)	(.001227, .002024)
20 R	.001599	(.001293, .001957)	(.001218, .002058)
100 R	.001230	(.000921, .001608)	(.00850, .001717)
500 R	.002761	(.002274, .003321)	(.002155, .003480)

It can be readily seen that the mutation frequencies for 0, 20, and 100 R all overlap. Only the frequency for 500 R is significantly different.

In another program, the difference of two frequencies is computed, and the Chi Square Significance Level (P) obtained

1) Experiments 1-130, (all data available to date):

<u>COMPARISON</u>	<u>LETHAL FREQUENCIES (%)</u>	<u>CHI SQUARE SIGNIFICANCE LEVEL (P)</u>
0 R vs. 20 R	.1590 vs. .1599	.967
0 R vs. 100 R	.1590 vs. .1230	.127
0 R vs. 500 R	.1590 vs. .2761	.000042
100 R vs. 500 R	.1230 vs. .2761	.000001

Since the 100 R treatment only began with experiment 34, we decided to have another set of comparisons by excluding data from experiments 1-33.

2) Experiments 34-130 only:

<u>COMPARISON</u>	<u>LETHAL FREQUENCIES (%)</u>	<u>CHI SQUARE SIGNIFICANCE LEVEL (P)</u>
0 R vs. 20 R	.1392 vs. .1630	.333
0 R vs. 100 R	.1392 vs. .1230	.492
0 R vs. 500 R	.1392 vs. .2746	.000006
100 R vs. 500 R	.1230 vs. .2746	.000001

The main difference for the first and second tabulation is that the difference between results from 0 and 100 R, which had a suggestively low significance level of .127 in the first tabulation, is now clearly non-significant.

In previous work with immature germ cells using high X-ray doses, the induced sex-linked lethal mutation rate was approximately 0.5% per 1000 R. Since we found a rather low mutation rate for our 500 R dose, we decided to test some higher doses within the present experimental setup. The high doses were given at the same intensities as the low doses (approximately 70 to 75 R per minute).

The results (uncorrected for controls) were as follows:

		% Leth. $\pm$ s.e.
3000 R	39 lethals in 2908 chromosomes tested	1.341 $\pm$.213%
6000 R	147 lethals in 5097 chromosomes tested	2.884 $\pm$.234%

While the number of tests are still small, one can make a rough estimate based on the 6000 R data. The induced portion for 6000 R is 2.725%. Assuming a linear relationship we would expect 500 R to induce one twelfth as much, or .227%. Since we only obtained an induced component of .117%, it seems at this point in our investigation, that we obtain only half as many induced lethals as expected on a linear model for a dose of 500 R.

Not much can be said at this time about the low lethal frequency found for 100 R, until much more data have been collected. The deviation from the control rate is more often than not in a negative direction; should this persist, it might point to an interesting biological phenomenon. Certainly, the lethal rate is not higher than expected, in contrast to Oftedal's results from spermatogonia irradiated in *Drosophila* embryos or larvae.

BREEDING SCHEME

P₀: Balanced stock furnishing virgin females:

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

P₁:

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

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:

One single F₁ daughter

$$\frac{y \quad \text{dow} \quad \text{---}}{y^{3ld} \quad w^{FM6} \quad B, \text{FM6}} \quad \text{"automatically virgin" (*)} \quad \times \quad \frac{y^{3ld} \quad w^{FM6} \quad B, \text{FM6}}{y^+} \quad \text{♂♂}$$

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.

12

STATEMENT OF TIME DEVOTED TO THIS PROJECT

The Principal Investigator, Seymour Abrahamson, has devoted 10% of his time during the academic year to these investigations. The Co-Principal Investigator, Helen Unger Meyer, has devoted her full time to work on this project. These proportions will remain the same for both during the remainder of this grant period.