MASTER

TECHNICAL PROGRESS REPORT

GENETIC EFFECTS OF LOW
X-RAY DOSES IN DROSOPHILA⁽¹⁾

(Period Covered: August 16, 1970 to August 15, 1971)

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I. INTRODUCTION

The work to be reviewed in this Annual Progress Report is a continuation of a large-scale investigation on the mutagenic effects of very low X-ray doses on the immature germ cells of *Drosophila melanogaster*. Our main objective is to determine the relationship of dose to the frequency of sex-linked lethal mutations induced in oogonial cells. Linearity of dose and mutation frequency for "one-track" mutations has been demonstrated for a wide range of X-ray doses, and for different germ cell stages; it was also found to be valid for very low doses when mature spermatozoa were irradiated. However, very little information is yet available for immature, metabolizing germ cells which are still capable of undergoing cell divisions.

It is entirely possible that a deviation from linearity may be found when gonial cells are irradiated with very low doses. This has been claimed by Oftedal who found that mutation frequencies induced by low doses in spermatogonia of *Drosophila* are higher than expected on a linear model. Our present experiments are trying to investigate this issue for oogonial cells.

II. EXPERIMENTAL PROCEDURES

An investigation of low dose effects is necessarily a long-term undertaking and changes in the experimental procedure are to be avoided, unless absolutely necessary. When we initially designed this experiment we tried to anticipate any future needs which might arise during the course of the work. The experimental procedures and the breeding technique for detecting

newly arisen sexlinked lethals in the progeny of irradiated females has been discussed in great detail in our initial grant proposal, and will again be presented in our Comprehensive Progress Report. Since no essential changes in procedure were made since last year's Progress Report was submitted, we include here again an outline of this procedure (see "Breeding Scheme") which gives all the essential points.

The X-ray doses used in this year's work have remained the same as during the previous year and are 0 R, 20 R, 100 R, and 500 R. Originally we had planned to add a 1000 R dose, but then decided not to dilute our efforts until the trends in our data might become more clear; there was also less technical help than we had hoped for. We did instead add a very high dose, 6000 R, in 4 experiments during this last year. Since we obtained much lower "induced" rates of sexlinked lethals than expected on a linear model, or on the basis of Oftedal's low dose results for spermatogonia, it became necessary to check the potential mutability of the experimental X-chromosome (y dow). This high dose of 6000 R was given over a period of 8 days in fractions of 3 X 2000 R, and at the same dose rate as the low doses. The results were reassuring (see "Results").

One other adjustment made during this past year concerned the distribution of the young virgin females used for irradiation (P_1 females) among the 4 dose groups in each experiment. Ideally the proportions should remain the same in each experiment, but the curiously low "induced" lethal frequencies found by us (though this is not at all a statistically significant fact) made

BREEDING SCHEME

P₀: Balanced stock furnishing virgin females:

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

P₁:

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

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

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

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.

some adjustments advisable. We have gradually changed from the initial proportions of 32% : 32% : 20.7% : 16.2% to the present distribution of 27% : 27% : 26% : 20% for doses of 0 R, 20 R, 100 R, 500 R, respectively.

Since gonial cells have a very low mutational response to X-rays and because of the low doses used, the vast majority of lethals detected in our experiments will be of spontaneous origin. Therefore it is of the utmost importance to minimize any conditions which might influence the spontaneous "background" mutation rate. We have mentioned the various precautions used in our experiments in the Renewal Proposal, and will also discuss the various points in the Comprehensive Report.

By far the most important precaution is the elimination of P_1 females which are already heterozygous for a pre-existing lethal in the experimental X-chromosome. As pointed out in the outline of the attached Breeding Scheme, this is achieved by a thorough inspection of the progeny from each individual P_1 female; all cultures which do not have a reasonable number of y dow F_1 males are eliminated from the experiment.

We also use the precaution of restarting the stock, which supplies the P_1 females for irradiation, every 6 months by reintroducing lethal-free X-chromosomes derived from non-lethal controls. Several routine precautions which serve to randomize some environmental factors have been developed during the course of the work, and have been described in this year's Renewal Proposal. They will be discussed in more detail in the

Comprehensive Report to be submitted shortly.

Finally, we want to repeat here our criteria for a sex-linked lethal, as used in these experiments. For cases of partial lethals to be included as "lethals" in our tabulations, we have set our cut-off point at 5% of expectation, and not at the more common 10% limit. The "expectation" is periodically determined from non-irradiated control cultures of good viability. We count as a sex-linked lethal mutation any case where the ratio of males with the experimental X-chromosome to females heterozygous for this chromosome is 5% or less in F_2 and in subsequent generations.

III. RESULTS

At the time of submitting last year's Progress Report we had completed 51 experiments; the first 33 included only X-ray doses of 0 R, 20 R and 500 R: starting with experiment 34, a 100 R dose was added.

During the past year we have continued to accumulate more data for these same four dose groups. In 38 additional experiments we have processed 136,580 more P_1 females, from which we were able to test 69,427 F_1 females for sexlinked lethals, each derived from a different P_1 female. To illustrate the range of variability of the lethal frequencies we will first present our new data (experiments 52 through 90) divided arbitrarily into 3 parts, each summarizing 9-10 consecutive experiments. Later on we will present the cumulative totals based on the entire data obtained so far.

<u>Exp. No.</u>	<u>Dose</u>	<u>No. Leth./Total tests</u>	<u>% Sexlinked Leth.</u>
52-70	0 R	8/8498	.094
71-80	0 R	9/5274	.171
81-90	0 R	7/5849	.120
52-90 totals		24/19621	.122
52-70	20 R	18/8678	.207
71-80	20 R	9/5402	.167
81-90	20 R	7/5931	.118
52-90		34/20011	.170
52-70	100 R	7/6297	.111
71-80	100 R	5/4789	.104
81-90	100 R	7/4775	.147
52-90		19/15861	.120
52-70	500 R	24/5568	.431
71-80	500 R	6/4161	.144
81-90	500 R	12/4205	.303
52-90		42/13934	.301
64,67, 68,70	6000 R	103/3653	2.820

The cumulative totals presented in last year's Progress Report were based on 51 completed experiments, with 57,644 effective F_1 tests (from 158,930 P_1 females processed). The data are here presented again, with the frequencies of sexlinked lethals and standard errors:

0 R, 58 lethals in 21,763 tests, $.267 \pm .035\%$
20 R, 33 lethals in 20,715 tests, $.159 \pm .028\%$
100 R, 14 lethals in 6,612 tests, $.212 \pm .057\%$
500 R, 24 lethals in 8,544 tests, $.281 \pm .057\%$

At the present writing we have completed 39 more experiments with these same doses, with a total of 127,061 effective F_1 tests (from 295,510 P_1 females processes). The cumulative summary for experiments 1 through 90 is as follows:

0 R,	82 lethals in 41,384 tests,	.198 ± .022%
20 R,	67 lethals in 40,726 tests,	.165 ± .020%
100 R,	33 lethals in 22,473 tests,	.147 ± .026%
500 R,	66 lethals in 22,478 tests,	.294 ± .036%

We have also included a very high X-ray dose, 6000 R (given in 3 fractions of 2000 R over a period of 8 days) in four of this year's experiments, in order to check on the potential mutability of our experimental X-chromosome (y dow). From 9,675 P₁ females treated with 6000 R we obtained 103 lethals in 3653 tests, a frequency of 2.820 ± .2736% sexlinked lethals, not corrected for controls. The frequency of corresponding controls was 4 in 2070, or .1927%.

Comparing the mean frequencies of sexlinked lethals based on the cumulative totals from experiments 1-51, with those from all experiments completed so far (experiments 1-90) we see that the control frequency has become lower, the frequencies for 20 R and for 500 R remained similar and that the frequency of the 100 R group now shows a lower value.

The data for 20 and 100 R still overlap the control rate; the frequency for 500 R is significantly above the control rate, but less so than projected on a linear model from higher doses. Extrapolating from data obtained with higher doses for gonial cells, we would expect an increase of approximately .25% above the control rate for 500 R. However, the frequency obtained for the high dose of 6000 R is within the expected range of approximately 3% induced sexlinked lethals, which

reassured us that the experimental chromosome has the potential ability to undergo mutation at an average frequency, when irradiated with higher doses. We plan to add 2 more dose points in next year's experiments, 1500 R and 3000 R, to relate the information gathered from the low doses with that obtained at higher doses. Our main effort will remain the investigation of the low dose range, for which many more data have to be accumulated before we will have any statistically meaningful answers.

STATEMENT OF TIME DEVOTED TO 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 the grant period.

NEW PUBLICATIONS RELATED TO THIS PROJECT:

Abrahamson, S., P. Gullifor, E. Sabol, and J. Voigtlander 1971
Induction of Translocations in Mature *Drosophila* Oocytes
over a Dose Range of 10-500 Roentgens of X-rays. Proc.
Natl. Acad. Sci. USA 68: 1095-1097.

Meyer, Helen U. and Seymour Abrahamson 1971 Preliminary Report
on Mutagenic Effects of Low X-ray Doses in Immature Germ
Cells of Adult *Drosophila* Females. (Abstract) Supplement,
Genetics 68: s44.