MASTER

TECHNICAL PROGRESS REPORT

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

(Period Covered: September 1, 1969 to August 15, 1970)

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INTRODUCTION

The objectives and methods of our investigations on the genetic effects of very low doses of X-rays on oogonial cells of Drosophila melanogaster have been described in last year's Progress Report. Since this experiment was designed to be a long-term study, in which the methods should remain essentially the same, this description is still valid. We will therefore include some portions of that report updated by minor changes and additions. A new section will be devoted to describing the most recent data and preliminary interpretation.

I. Purpose of Investigations

Most previous research on the genetic effects of low X-ray doses in *Drosophila* and other higher organisms has been carried out on the highly radiation-sensitive chromosomes of mature, or nearly mature germ cells. A linear relationship between dose and mutation rate was found. This work and the relevant literature has been discussed in detail in our initial Research Proposal.

Very little is known about the dose kinetics, for low X-ray doses, for the mutability of chromosomes in immature, metabolizing germ cells which have extremely low X-ray sensitivity. Since these stages persist for long periods of the life span of many higher organisms, such information would be of great practical and theoretical importance.

We therefore decided to embark upon a large-scale project of testing X-chromosomes from oogonia of Drosophila melanogaster for recessive lethal mutations using doses of 500 R, 20 R, and 0 R (control group) in our initial experiments (1968-1969). A third dose point, 100 R was added in 1970.

II. Experimental Methods

Oogonial cells are irradiated in young adult virgin females 25 hours or less after eclosion. Three X-ray doses (20 R, 100 R and 500 R) are given at the rate of approximately 70 R per minute with a G.E. Maxitron-300 machine; the 500 R treatment is divided into fractions of 200 R, 80 R, 20 R, and another 200 R dose; the 100 R dose is given as 80 R plus 20 R with the 20 R dose group inserted at the proper time. The time needed for each interruption is about 30 seconds.

The genetic technique of testing for newly arisen recessive lethals in X-chromosomes in the germ cells of the treated females (P_1) has been outlined and discussed in detail in our initial Research Proposal (1968). A condensation of the procedures for screening for sex-linked lethals is given on the following page (Breeding Scheme). As shown, only two minor modifications were made: The post-irradiation brood period for P_1 females, - with the purpose of depleting their supply of post-gonial germ cells, - was shortened from 16 to 13 days; at the end of this period these fertilized P_1 females are now cultured individually in vials without the presence of males. This eliminates the need to remove the fertile P_1 -males before emergence of the next (F_1) generation, when only unfertilized females are desired, which are otherwise provided by the special genetic constitution of the P_1 -females.

BREEDING SCHEME

P₀: Balanced stock furnishing virgin females:

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

P₁:

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

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:

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

Cultures raised at 25°C

F₂: Scored for y dow males; absence or scarcity indicates viability mutation.

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.

III. Special Precautions

Special precautionary measures are taken to exclude from further testing the offspring of P_1 -females which could be suspected of bearing a pre-existing lethal in the experimental (y dow) X-chromosome. The F_1 offspring from each individually cultured P_1 is inspected, without etherization, for presence of at least two y dow males. All other cultures are discarded, including many cultures with only a small number of F_1 .

This elimination procedure reduces the number of F_1 tested considerably, but we feel that it is of the utmost importance for a low-dose study of gonial cells with very low X-ray mutability, to determine the background rate of spontaneous mutations as accurately as possible.

Since oogonia are dividing cells, we may expect that clusters of mutations of identical origin (spontaneous or induced) are frequently recovered when testing F_1 -females for lethals. This would lead to a large statistical error, because the number of F_1 -tests for lethality would be greater than the number of oogonial chromosomes actually sampled. We therefore test only one single X-chromosome from each lethal-free experimental P_1 -female to avoid both a much larger than necessary statistical error and the additional work required of maintaining the families of a single female and conduct subsequent allelism tests.

While the majority of recessive lethals in the X-chromosome are characterized by complete absence of the critical class of males (in our experiment, y dow males), some are borderline cases

in which a few such males survive, the so-called partial lethals as well as severe detrimentals. For the purpose of these experiments we have defined a lethal as a case where the frequency of males with the experimental X-chromosome lies between 0 and 5% of the mean frequency found in control cultures with an obviously good viability of this class of males. Confirmation tests with progeny counts for accurate classification are made for all cases of suspected lethals. Special Y chromosomes carrying duplications for the X-chromosome regions of y^+ , w^+ , v^+ and mal^+ are utilized to assign some of the lethals to these specific regions.

IV. Results

a) First results (1968-1969)

At the time of submitting last year's report, 23 irradiation experiments had been completed. About 65,000 P_1 virgin females were treated, mass-mated in bottles and brooded for at least 13 days. At the end of this period some 31,300 fertilized females were still alive and were individually cultured in vials. Only 11,760 of these cultures passed our stringent criteria for absence of a pre-existing lethal in the y dow chromosome and were accepted for further testing.

About 11,000 X-chromosomes had then been effectively screened for lethal mutations, each derived from a different P_1 -female. Results from experiments 1-23 were:

0 R,	10 lethals in 4647 tests,	.2152%
20 R,	9 lethals in 4677 tests,	.1924%
500 R,	5 lethals in 1664 tests,	.3005%

b) New results (1969-1970)

Ten more experiments have since been added for these same

two X-ray doses. The cumulative totals (including the data just mentioned), based on 106,694 P_1 females processed and on 27,551 effective F_1 -tests are, for experiments 1-33:

0 R,	30 lethals in 12,334 tests,	.2432%
20 R,	17 lethals in 11,537 tests,	.1474%
500 R,	11 lethals in 3,680 tests,	.2989%

Beginning with experiment 34 another dose point, 100 R, was added. At this writing 51 experiments have been completed and some 52,250 additional P -females processes with 30,086 effective F -tests. Results from experiments 34-51 are:

0 R,	28 lethals in 9429 tests,	.2970%
20 R,	16 lethals in 9181 tests,	.1743%
100 R,	14 lethals in 6612 tests,	.2117%
500 R,	13 lethals in 4864 tests,	.2673%

Summarizing all experiments completed to date, we obtain for experiments 1 through 51 with 158,930 P_1 -females processed and 57,637 effective F_1 - tests the following frequencies and standard errors:

0 R,	58 lethals in 21,763 tests,	.2665 $\pm$.0349%
20 R,	33 lethals in 20,718 tests,	.1593 $\pm$.0277%
100 R,	14 lethals in 6,612 tests,	.2117 $\pm$.0565%
500 R,	24 lethals in 8,544 tests,	.2809 $\pm$.0573%

All frequencies of sex-linked lethals for these four groups are similar, at least within a range of the twofold standard errors. No effect of the X-ray treatments can as yet be

detected, though the number of F_1 tests for the two higher doses are still quite small. We might tentatively say that our data at the present time do not encourage the assumption that the low X-ray mutability found for all gonial cells with higher doses in other work might be a consequence of selection against an especially reiradiation-sensitive stage of gonial cell. A vast amount of data will have to be accumulated before we can make any decision about the question of linear dose kinetics operating or not operating at very low X-ray doses (i.e. over a range of 20-500R) in oogonial cells of *Drosophila*.