GENETIC EFFECTS OF LOW X-RAY DOSES

Progress Report

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ABSTRACT

Our current large-scale investigation aims to determine dose kinetics for immature metabolizing germ cells (oogonia) of *Drosophila* for the low dose range (20 to 500R). Sporadically, experiments have included higher doses (3000 and 6000R); this last year an intermediate dose, 1500R, was added.

The frequency of new sex-linked lethals is used to measure mutagenicity; parent-females with pre-existing lethals are excluded. Only one single daughter (F_1) from each experimental female is tested, avoiding clusters of mutations of identical origin. Strict numerical standards are used for classification and retesting of suspected lethals.

To date, over 428,000 X-chromosomes have been successfully analyzed, from 228 separate experiments. Mutation frequencies for low doses up to 200R still overlap the control range; only 500R gives a significantly higher value. However, our data suggest that the per-roentgen yield of mutations for 500R is much lower than expected by linear projection from high-dose data for gonial cells.

Summarizing all data, the following lethal frequencies (in percent) with standard errors were obtained (not corrected for controls): 0 R (control), $.137 \pm .011$ (109, 386 tests); 20R, $.151 \pm .015$ (67, 553); 100R, $.128 \pm .012$ (89, 536); 200R, $.163 \pm .017$ (56, 429); 500R, $.266 \pm .018$ (83, 958); 1500R, $.847 \pm .123$ (5, 548); 3000R, $1.438 \pm .127$ (8, 830); 6000R, $2.829 \pm .193$ (7387).

I. PURPOSE OF THE INVESTIGATION

We have continued our large-scale investigation of the dose kinetics for the induction of recessive sex-linked lethals in immature germ cells of *Drosophila* (oogonia) by X-rays. Linear relationship for dose and mutagenic effect has been demonstrated for mature germ cells, from high down to very low doses. This linearity principle forms the basis for estimates of radiation risks at present; however the principle of linearity might not be valid for the low dose range when immature, metabolizing germ cells are treated.

Oftedal (1964) irradiated spermatogonia of late *Drosophila* embryos with low X-ray doses, and his results did not agree with the linearity principle. He obtained a much higher frequency of induced sex-linked lethals for acute doses up to 155R, but no increase over the control rate with 300R.

Since immature germ cells, as stem cells, can persist for a long time during the life of an organism and thus may accumulate mutations, it is of greatest importance that a large-scale study be made to get further information on the dose kinetics of gonial cells for the low dose range.

Our own experiments were designed to expand and complement Oftedal's data; we have been irradiating oogonia of adult *Drosophila* females with doses up to 500R, and analyzed their progeny for newly arisen sex-linked lethal mutations. During the course of our work it became desirable to check the mutational potential of the X-chromosome used in our study by giving higher doses on some occasions.

II. EXPERIMENTAL PROCEDURES

The experimental procedures have remained essentially the same during the entire course of our long-term investigation of the low-dose effects in immature germ cells. The only changes that were necessary from time to time were the addition of more X-ray doses. During the current grant period we have included an intermediate dose (1500R) in our experiments, and we have discontinued data collection for 20 and for 6000R.

At present the following X-ray doses are given contemporaneously in each experiment: 0, 100, 200, 500, and 1500R; sometimes, when only a smaller scale experiment can be done, the doses are 0, 500, 1500R, and sporadically also 3000R. All irradiations are with 250 Kv X-rays, at a dose rate of 75-85R/min, from a G.E. Maxitron-300 hospital type X-ray machine.

Young virgin females (P_1) are irradiated, then mated immediately and brooded intensively for 13 days. After this period the fertilized females are cultured individually in vials. P_1 -females with a pre-existing lethal can be recognized by their offspring, and such cultures are discarded. Only one single daughter (F_1) from each of the P_1 -females is tested for possible presence of a new lethal; thus clusters of mutations of identical origin can be avoided. Strict numerical standards are employed for classification and re-testing all cases of suspected new lethals.

Our special breeding scheme for detecting new sex-linked

lethal mutations in irradiated females has been discussed many times before in detail. A diagrammatic outline of this breeding technique is again included in this report. The P_1 -females are collected as virgins, kept overnight at cool temperature to retard egg maturation and irradiated when they are 16 to 25 hours old. The P_1 -females are heterozygous for our "experimental" X-chromosome marked by yellow body (y) and by downy bristles (dow); the latter is also a male sterilizing gene. This male-sterile mutation in one of the female's X-chromosomes, and a lethal deficiency in the balancing inversion chromosome (covered in the stock by a special, so-called sc^8 -Y chromosome) make it possible to obtain only unfertilized females in the F_1 -generation. More details of the breeding procedure are given in the diagram.

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

Different investigators have used various criteria for lethality. In some cases, only "complete" lethals are counted, in others, also partial lethals. In this present investigation we count as lethal mutations only those 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. It is possible for us, through our computer program, to use other definitions for a lethal (no survivors, or $\leq 2\%$, or $\leq 10\%$ surviving) and obtain the frequencies using other criteria. This method enables us to compare our data to those of other investigators who use different standards.

III. RESULTS TO DATE

Since we submitted our last report (July 1975) we have conducted 53 more experiments from which over 104,000 X-chromosomes could successfully be tested for new lethals. Each chromosome came from a different P_1 -female. Our main effort during this year has been to get more data for the low-dose range, especially the 200 R dose. The lowest dose, 20 R, was phased out, and we did not use the highest dose of 6000 R at all. An intermediate dose, 1500 R, was added only since January 1974. Some more data were obtained for 3000 R, but they are not included here in this survey of new results, since tests were not complete at the cut-off point for this present report, which is experiment 228.

The new results, from experiments 176-228, are as follows (frequencies are uncorrected for controls):

DOSE	No. Leth./Total Tests	%LETH. $\pm$ S.E.
OR	27/21,857	.124 $\pm$.024
20R	0/867	.000
100R	31/25,172	.123 $\pm$.022
200R	41/27,403	.150 $\pm$.023
500R	48/23,266	.206 $\pm$.030
1500R	47/5,548	.847 $\pm$.123

This segment of data by itself is not very meaningful, but confirms the general trend: Among the low-dose treatment, only 500 R gives an elevation above the control frequency. The results for 1500 are not extensive enough to judge whether the induced frequency is more in line with expectations based on our results from 500 R, or from the high dose data.

Pooled data from experiments 1-228 are presented in Tables 1-2. The first two tables give only the results from the low dose treatments, arranged in 5 groups of consecutive experiments: In Table 1 the data are given separately for each section; in Table 2 we have cumulative totals based on the same data. The purpose of dividing the data into several groups is to show how much variability there is in the results from different doses.

Table 3 compares the mutation frequencies of control (0 R) and 500 R with those obtained from the intermediate 1500 R treatment and our 2 high doses. In this table the frequencies for 0 and 500 R are based on data from experiments 64-228, because only during this period were the higher dose treatments given.

In Table 4 we present again the results from low-dose treatments, but restricted to experiments 34-228, since the 100 R dose had not yet been included in experiments 1-33.

The low-dose data for experiments 34-228 (Table 4) were subjected to a Chi-square significance test, also by computer program.

Paired comparisons of the mutation frequencies obtained with various doses gave the following P-values:

O R versus 20 R	P = .1477
O R versus 100 R	P = .7705
O R versus 200 R	P = .0551
O R versus 500 R	P = .000000
100 R versus 20 R	P = .2431
100 R versus 200 R	P = .1038
100 R versus 500 R	P = .000000
200 R versus 500 R	P = .000066

P- values for the comparison of O R versus 20 and 100 R indicate no statistically significant difference; that all three mutation frequencies overlap was already shown by the standard errors. The P-values obtained for the comparisons for a dose of 500 R versus 0 and 100 R and 200 R confirm that there is a highly significant elevation of the mutation frequency with 500 R.

The P-value of .0551, obtained in the comparison 0 versus 200 R suggests that further data collection might possibly show that 200 R increases mutations above the control rate. At present we are not certain that it does, since the two frequencies still overlap within the range of their twofold errors (see Table 1, Summary; also Table 4).

The fact that we cannot demonstrate an increase over the spontaneous mutation frequency for doses of 20 and 100 R, does not mean that such an increase does not exist; but if it does, it must be of an extremely low order of magnitude.

Ideally, all low-dose points under consideration should be included in every experiment. Comparison with contemporary controls is crucial, because almost all mutations found from 20 and 100 R must be of spontaneous origin.

We therefore compiled all data on mutation frequencies for

0, 20 and 500 R from all experiments in which these 3 doses were present:

<u>Dose</u>	<u>No. Leth./ Tot. Tests</u>	<u>% Leth. $\pm$ S.E.</u>
O R	112 / 73,132	.153 $\pm$.015
20 R	102 / 67,553	.151 $\pm$.015
500 R	138 / 49,051	.281 $\pm$.024

This compilation shows how very close the mutation frequency with 20 R is to that of the control and illustrates why we have not continued 20 R exposures, but instead put the main emphasis on obtaining more data for 100, 200 and 500 R.

Mutation frequencies with a dose of 500 R were .260%, .266%, and .264% for the different versions of pooled data (Tables 1, 3 and 4). Correcting for corresponding control rates, which show relatively greater fluctuation, we obtain these rough estimates of induced frequencies: .129% (Table 1), .153% (Table 3) and .140% (Table 4).

Our high dose treatments with 3000 and 6000 R (Table 3) give us a projected value of .222% or .226% sex-linked lethals per 500 R on a linear model. This is in reasonable agreement with previous data from high dose treatments of gonial cells, .50% for 1000 R. Our slightly lower rate might be due to more stringent criteria for defining lethality.

We conclude therefore from the present status of our data that a single dose of 500 R induces only approximately 63% as many sex-linked lethal mutations as expected from high dose treatments on a linear projection. This means that linear dose kinetics is not operating over the entire range from low to high X-ray doses in oögonia of *Drosophila*.

It will be of great importance to do more refined statistics when more data for 200 and for 1500 R will be available, which we hope will be accomplished during the coming year.

RECENT PUBLICATION

Seymour Abrahamson, Michael A. Bender, Alan D. Conger 1973 Uniformity of Radiation-induced Mutation Rates among different Species. Nature, vol. 245, pp. 460-462.

<u>DOSE:</u>	<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)	.222 $\pm$.056 (7,199)	.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. 176-228	.124 $\pm$.024 (21,857)	0.0 (867)	.123 $\pm$.022 (25,172)	.150 $\pm$.023 (27,403)	.206 $\pm$.030 (23,266)
Summary Exp. 1-228	.137 $\pm$.011 (109,386)	.151 $\pm$.015 (67,553)	.128 $\pm$.012 (89,536)	.161 $\pm$.017 (56,429)	.266 $\pm$.018 (83,958)

Table 1: Frequencies of new sex-linked lethals with standard errors, from oogonia irradiated with low X-ray doses. Total number of X-chromosomes tested in parentheses.

DOSE:	0R	20R	100R	200R	500R
Exp. No.	%l. $\pm$ s.e.	%l. $\pm$ s.e.	%l. $\pm$ s.e.	%l. $\pm$ s.e.	%l. $\pm$ s.e.
1-48	.251 $\pm$.035 (20,305)	.165 $\pm$.029 (19,346)	.200 $\pm$.060 (5,502)	--	.240 $\pm$.055 (7,919)
1-90	.197 $\pm$.022 (42,130)	.164 $\pm$.020 (40,826)	.142 $\pm$.025 (22,473)	--	.292 $\pm$.036 (22,576)
1-130	.159 $\pm$.016 (64,780)	.160 $\pm$.017 (58,774)	.123 $\pm$.017 (43,094)	.222 $\pm$.056 (7,199)	.276 $\pm$.026 (40,566)
1-175	.141 $\pm$.013 (87,529)	.153 $\pm$.015 (66,686)	.131 $\pm$.014 (64,364)	.172 $\pm$.024 (29,026)	.288 $\pm$.022 (60,692)
1-228	.137 $\pm$.011 (109,386)	.151 $\pm$.015 (67,553)	.128 $\pm$.012 (89,536)	.161 $\pm$.017 (56,429)	.266 $\pm$.018 (83,958)

Table 2: Cumulative frequencies of new sex-linked lethals, with standard errors, grouped as in Table 1. Total number of X-chromosomes tested in parentheses.

X-RAY DOSE	NO. LETH./TOT. TESTS (% LETH. $\pm$ S.E.)	EXCESS OVER CONTROL RATE
0 R	87/81,373 (.107 $\pm$.010)	— —
500 R	187/71,814 (.260 $\pm$.019)	.153%
1500 R	47/5548 (.847 $\pm$.123)	.740%
3000 R	127/8830 (1.438 $\pm$.127)	1.331%
6000 R	209/7387 (2.829 $\pm$.193)	2.722%

Table 3: Frequencies of new sex-linked lethals, with standard errors, from oogonia irradiated with 500 R and with some higher doses. Data for controls and for the dose of 500 R based on experiments 64-228 (see text for more details).

X-RAY DOSES	NO. LETH./TOT. TESTS (% LETH. $\pm$ S.E.)	EXCESS OVER CONTROL RATE
0 R	120/97,052 (.124 $\pm$.011)	— —
20 R	85/56,019 (.152 $\pm$.016)	.028%
100 R	115/89,536 (.128 $\pm$.012)	.004%
200 R	91/56,429 (.161 $\pm$.017)	.037%
500 R	212/80,178 (.264 $\pm$.018)	.140%

Table 4: Frequencies of new sex-linked lethals from oogonia. Summary of all data from experiments 34-228, i.e. omitting the first 33 experiments which did not yet include treatments with 100 R.

BREEDING SCHEME

P₀: Balanced stock furnishing virgin females:

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

$$\begin{array}{c} \text{P}_1: \\ \text{Virgin} \quad \frac{Y \text{ dow}^{**}}{\text{Df, Basc}^-} \quad \text{♀♀} \quad \times \quad \frac{y^{3\text{ld}} w^{\text{FM6}} \text{ B, InsFM6}}{Y^+ \text{ (normal Y)}} \quad \text{♂♂} \end{array}$$

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:

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

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.