GENETIC EFFECTS OF LOW X-RAY DOSES

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ABSTRACT

Experiments on dose-kinetics of X-ray induced sexlinked lethal mutations in Drosophila oogonia were continued. A wide range of doses was tested, with special emphasis on the low-dose range (20-500R). This year more data were added for 0 R, 200, 500, 1500R and some high doses. Oogonia of adult females were irradiated, and only one daughter from each experimental parent-female was analyzed for a new lethal in her maternal X-chromosome. Thus no clusters of mutations of identical origin are encountered.

Our extensive accumulated data do not support the linearity principle, firmly established for recessive mutations induced in mature spermatozoa for high down to very low X-ray doses. To account for our oogonial data we have proposed a mathematical model describing the mutational yield as $Y = C + \alpha D + \beta D^2$; the control frequency C and the coefficients α and β are obtained from the data by weighted regression analysis.

This linear-quadratic model suggests that recessive mutations, like chromosome aberrations, result from both one-track and two-track events, with the latter playing an increasingly important role at higher doses. Accordingly, we would expect that acute and fractionated irradiations for equivalent high doses would give different mutational yields. We are at present involved in testing this prediction.

INTRODUCTION

We have continued the investigation of dose-kinetics for X-ray induced sexlinked lethals in Drosophila oogonia. This cell stage has an extremely low mutational response to ionizing radiations. Doses of 20-500R, which we consider low doses for this germ cell stage, would be approximately equivalent to 2-50R in human gonads.

Though our main effort was exploration of the low-dose range, it soon became necessary to test several higher dose points. We now have accumulated extensive data for a wide range of doses. The lethal frequencies obtained by us do not show linear proportionality to dose, over the range of doses studied, as in the case of irradiation of mature spermatozoa.

Last year we proposed a model of dose kinetics which seems to satisfy our data quite well. Our data are most simply accounted for by the quadratic equation $Y = C + \alpha D + \beta D^2$ where C is the control frequency and α and β are the coefficients of the dose D. We have obtained the values for $C = 1.30 \times 10^{-3}$, $\alpha = 2.56 \times 10^{-6}$, $\beta = 7.56 \times 10^{-10}$, from our data by weighted regression analysis (weighted by the inverse of the binomial variance). Our data and the analysis provide no evidence for a

higher mutation rate per R at lower doses (as reported by Oftedal), and obviates the need to invoke the ad hoc hypothesis of repair of premutational damage at lower doses. Indeed the quadratic analysis suggests that mutations, like chromosome aberrations, result from one-track and the interaction of two track events, the latter playing an increasingly important role at the higher doses. Accordingly, we would expect that acute and fractionated irradiations for equivalent high doses would give different mutational yields. We are at present involved in testing this prediction.

EXPERIMENTAL PROCEDURES

The genetic scheme used during the entire course of our investigation was described in previous reports; it has remained unchanged. A brief outline with diagram is again attached to this report.

The experimental P_1 -females are collected as virgins, kept at cool temperature on sugar-agar medium overnight, and are irradiated 16-25 hrs. after eclosion. They are then mated immediately with an excess of males and brooded intensively until day 13 after irradiation. At this time the fertilized females are cultured individually (without males). Those heterozygous for a pre-existing lethal can be recognized by their offspring, and such cultures are discarded. We proceed by testing only one single F_1 -daughter from each treated or control P_1 -female for a possible new lethal in her maternal X-chromosome. Thus clusters of lethals of identical origin are avoided. Strict numerical standards are

employed for classification and retesting all cases of suspected new lethals.

Different investigators have used various standards for defining a lethal mutation. In this investigation we count as lethal mutations only those cases where the ratio of males with the experimental X-chromosome (y dow) when compared to their heterozygous sib-females is less than or equal to 5% of expectation. This "expectation" is empirically determined by periodic counts of the various classes of progeny from untreated control cultures with obvious good viability. Through our computer program we can summarize the data for different criteria (no survivors, or 2%, or 10% surviving). This enables us to compare our results with those obtained by other investigators using different standards for lethality.

A General Electric Maxitron-300 hospital type X-ray machine was used at 250 Kvp, 20 ma. The dose-rate was 70-85R/min for 20, 100, 200, 1500, 3000R and even for 6000R (divided into 3 fractions of 2000R each given over a week's time).

During the current year, when we began to give high doses in a single acute treatment, we increased the dose rate to 470-485R/min, so that the time span necessary to administer the highest dose tried (9000R) would not exceed 20 minutes, the time during potential breaks stay open. Doses given at this higher rate were 4500, 6000, 7500 and 9000R.

RECENT RESULTS

During the current grant period we have completed 46 more radiation experiments, with over 85,800 more X-chromosomes tested for newly arisen lethals. Most of these were for low doses, and some were for 1500 and 3000R. All these exposures were at a dose rate of approximately 80 R/min. The new data are:

Dose (R)	No. Leth./Total Tests	% Lethals
0	31/19,418	.1596
200	44/19,050	.2310
500	48/18,267	.2635
1500	118/16,857	.7005
3000	67/3,521	1.9029

Though not meaningful by themselves, these new results are in line with our previously accumulated data. (A summary of all data is presented in Table 1).

As next phase of our investigation we have begun experiments using high doses (dose-rate approximately 480R/min), to compare the effects of acute versus fractionated irradiations of oogonia. These experiments are still in pilot stage. The first objective was to find one or two suitable high doses which, when given acutely, would not cause too much sterility in the treated females. 9000, 7500, 6000 and 4500 R were tried, and the latter two doses found practicable. At present we give one acute treatment of 6000 R

and the same total dose in installments, 2 x 3000R and 4 x 1500R. All high dose irradiations are at a dose rate of 470-485 R/min., and the 1500R fractions are delivered at approximately 80 R/min. Exposure time for each treatment is less than 20 minutes.

These new data so far are:

Dose (R)	No. leth./Tot. tests	Lethals $\pm$ s. e.
1 x 9000 R	49 leth./1082	4.53% $\pm$.63%
2 x 4500	62 leth./1403	4.42% $\pm$.55%
1 x 7500	84 leth./2063	4.07% $\pm$.44%
1 x 6000	182 leth./4146	4.39% $\pm$.32%
4 x 1500	46 leth./1317	3.49% $\pm$.51%

A comparison of observed and expected mutational yields from 6000R given in different regimes is presented in Table 4.

These preliminary results seem to indicate that for doses from 6000R to 9000R the sex-linked lethal frequencies reach a plateau at 4 or 5%. Apparently oogonia with a greater load of mutations fail to proliferate, or to mature.

An acute dose of 7500R causes about 60% sterility in the treated females, 9000R (acutely) 83%. 6000R with only 15% sterility seems to be a suitable high dose for our purpose, and the remainder of this year will be devoted to investigate mutation frequencies for this dose given acutely and when fractionated. Whether a total dose of 4500R will also be investigated will be decided when tests for this dose point, now underway, are completed.

ACCUMULATED DATA

The data accumulated during the entire course of this investigation are presented in Tables 1-4, arranged in various ways. Table 1 lists the frequencies of newly arisen sex-linked lethals for X-ray doses from 0 to 6000 R based on pooled data obtained over almost 8 years. The frequencies are not corrected for control rates.

Each of the 326 radiation experiments have always included, in addition to a control group, contemporaneous irradiations with several different doses; however not all dose groups were present at one time. Table 2 is a pairwise comparison of mutation frequencies obtained for each dose group and its contemporaneous controls, or those obtained over the same time span. The Chi-Square significance (P) shows that doses of 200 and 500 R and higher cause mutation frequencies to rise significantly above control level. We have now discontinued data collection for the low dose range.

Table 3 gives the observed lethal frequencies and those expected on the basis of the linear-quadratic model. The fit is remarkably good.

Table 4 presents some old (3 x 2000 R) and some recent data for a total dose of 6000 R if given acutely and in a fractionated regime. This comparison is still based on too few data and is only tentative, but it does not contradict predictions from the proposed model.

DISCUSSION

In our previous review and in our published presentation (1976) we pointed out that the dose-frequency relationship obtained at that time could not support a linear interpretation throughout the dose range covered. Since that time we have obtained data at still higher doses. Reanalysis of the combined data again reinforce the view that the quadratic equation provides the best fit to the data (see Table 3) whereas a weighted linear regression cannot accomodate the yields obtained at all doses. Moreover, from the data for high doses with various regimes obtained so far we observe that fractionated treatments (3×2000 R) give a significantly reduced yield of mutations when compared to a single acute treatment 6000 R. The reduced yield obtained by a different fractionation regime, 4×1500 R is in the direction predicted by the quadratic model, but because of the relatively small sample size and correspondingly larger standard error, the data are not yet significantly different from the unfractionated treatment. Further accumulation of data should provide a further test of the model.

Our new high dose data are also comparable to those obtained by other investigators with other systems, mouse, tradescantia etc., in which a saturation point or plateau point is reached implying that cell killing selectively removes the mutant cells from the treated population. If killing and mutation both result from chromosome aberrations then it is not surprising to find such a plateau. Our data suggest that single acute doses of 7500 and

9000 R show no further increase in mutation incidence than that produced by 6000 R (see page 6) - Again the data are not yet sufficiently extensive, but they are at present consistent with the view that the maximum mutation rate is reached at 6000 R.

Concerning the very low dose portion of the curve we can now show that a dose of 200 R has produced a significant increase in mutation rate above the control rate. Based on the α coefficient of mutation rate by either a linear or quadratic model it becomes apparent that we cannot demonstrate a significant difference at doses of 100 R or lower from the observed control rate .14% lethals. The dose of 100 R will theoretically contribute 1 additional lethal per 10,000 tests to the control, or 10 per 100,000 tests (we have observed 6). We see no further need to expend effort in this dose range. We note however, that since our system is unable to discriminate a significant increase at this dose range for more than 10^5 tests performed, does not imply that a threshold exists below 200 R. Such an interpretation, as has been suggested by others, would appear to be inconsistent with a rational understanding of radiobiology. There is an additional importance to this low dose (20 and 100 R) data: it provides no basis for the interpretation that a higher mutation rate per R exists at low than at high doses. Indeed our experiments were designed to test the so-called Oftedal (1964) model which suggested such a phenomenon.

The *Drosophila* system appears to us to be a pertinent system for elaborating mutation-dose response relationships. It now

appears that a simulation of much of the same phenomenon observed in mouse, tradescantia and neurospora experiments has been accomplished. A quadratic response is indicated for acutely delivered high doses of irradiation. A significant dose-rate response is observed (Muller, 1962) only for those doses which have a substantial 2-hit (quadratic) contribution. No dose rate effects are observed when the doses are in the linear (1-track) range of the mutation curve, Purdom (1962). Dose fractionation results in a reduced mutation yield relative to a single acute treatment again, only if the single acute dose has a significant β or 2-track contribution. An apparent saturation dose has been observed as in mouse specific locus studies on spermatogonia and tradescantra somatic mutations.

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BREEDING SCHEME

P₀: Balanced stock furnishing virgin females:

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

P₁:

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

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:

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

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.

Table 1

FREQUENCIES OF SEXLINKED LETHALS, ALL DATA POOLED

Dose (R) *	No. Lethals	% Leth. $\pm$ s.e.
	No. Tot. Tests	
0	203/143,796	.141 $\pm$.010
20	102/ 67,553	.151 $\pm$.015
100	141/102,491	.138 $\pm$.012
200	165/ 92,685	.178 $\pm$.014
500	318/118,169	.269 $\pm$.015
1500	341/ 45,534	.749 $\pm$.040
3000	255/ 17,023	1.498 $\pm$.093
6000	182/ 4,146	4.39 $\pm$.317
3 X 2000R**	209/ 7,387	2.829 $\pm$.193

* Dose rate 70-85 R/min, except 6000R at 480 R/min

** During the period of 1 week

Table 2

COMPARISON OF SEXLINKED LETHAL FREQUENCIES FROM IRRADIATED
OOGONIA AND CORRESPONDING CONTROLS

Comparison		No. Lethals	Chi-Square	
No. Exp.	Dose (R) *	Total Tests	% Leth. $\pm$ s.e.	Significance (P) of difference
147	20	102/67,553	.1510 $\pm$.0149	.9175
	0	112/73,132	.1531 $\pm$.0145	
224	100	141/102,491	.1376 $\pm$.0116	.5044
	0	135/106,336	.1270 $\pm$.0109	
164	200	165/92,685	.1780 $\pm$.0138	.0043
	0	105/84,080	.1139 $\pm$.0138	
317	500	318/118,169	.2691 $\pm$.0151	<<.001
	0	203/143,796	.1412 $\pm$.0099	
77	1500	341/45,534	.7489 $\pm$.0404	<<.001
	0	100/78,799	.1294 $\pm$.0133	
35	3000	255/17,023	1.4980 $\pm$.0931	<<.001
	0	100/78,899	.1267 $\pm$.0127	

*Dose rate: 70-85 R/min

Table 3

OBSERVED AND COMPUTED FREQUENCIES OF X-RAY INDUCED
SEX-LINKED LETHAL MUTATIONS (ACUTE DOSES)
DATA POOLED FROM 326 EXPERIMENTS

X-ray Doses*	Mutation Frequencies	
	Observed	Computed**
0R	$.14 \times 10^{-2}$	$.13 \times 10^{-2}$
20R	$.15 \times 10^{-2}$	$.14 \times 10^{-2}$
100R	$.14 \times 10^{-2}$	$.16 \times 10^{-2}$
200R	$.18 \times 10^{-2}$	$.18 \times 10^{-2}$
500R	$.27 \times 10^{-2}$	$.28 \times 10^{-2}$
1500R	$.75 \times 10^{-2}$	$.69 \times 10^{-2}$
3000R	1.50×10^{-2}	1.58×10^{-2}
6000R	4.39×10^{-2}	4.39×10^{-2}

*Dose rate 70-85 R/min, except 6000R at approximately 480 R/min.

**Based on model: $Y = C + \alpha D + \beta D^2$, where $C = 1.30 \times 10^{-3}$,
 $\alpha = 2.56 \times 10^{-6}$, $\beta = 7.56 \times 10^{-10}$.

Table 4

MUTATIONAL YIELDS FROM ACUTE AND FRACTIONATED IRRADIATIONS

Doses (R)	No. leth./Tot. tests	% Observed	% Computed*	Notes
6000	182/4146	4.39 ± .32	4.39	
3 x 2000	209/7387	2.83 ± .19	2.57	P<.01
4 x 1500	46/1317	3.49 ± .51	2.35	need more data

* Model: $Y = 1.31 \times 10^{-3} + 2.56 \times 10^{-10} D + 7.56 \times 10^{-10} D^2$

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. 1976 Mutation Process at Low or High Radiation Doses in Biological and Environmental Effects of Low-Level Radiation. International Atomic Energy Agency - Vienna, Vol. 1: 3-8.

Abrahamson, S. and H. U. Meyer. 1976 Quadratic Analyses for Induction of Recessive Lethal Mutations in Drosophila Ogonia by X-irradiation. International Atomic Energy Agency - Vienna, Vol. 1: 9-18.