MASTER

GENETIC EFFECTS OF LOW X-RAY DOSES

Progress Report

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ABSTRACT

A linear-quadratic model of dose-kinetics was proposed by us for X-ray induced recessive lethal mutations in oogonia of *Drosophila*. From this it should follow that at higher total doses fractionation treatments should give a lower yield of mutations than an equivalent acute exposure.

A dose of 6000 R, given acutely and in 3 different fractionation regimes gave results in the expected direction for 2 x 3000 R, and a significant decrease for 3 x 2000 R and for 4 x 1500 R fractionations. We have also performed more irradiations with acute doses of 3000, 4500, and 6000 R, to strengthen the data for computation of the model equation describing dose-kinetics for 250 Kvp X-rays under the conditions of our experiment. This model, for a mutational yield Y at dose D is $Y = 1.279 \times 10^{-3} + 2.667 \times 10^{-6} D + 7.159 \times 10^{-10} D^2$ on the basis of our present data.

PURPOSE OF THE INVESTIGATION

Extensive data have been collected by our group on the relationship of X-ray dose and the frequency of sex-linked lethal mutations induced in immature germ cells (oogonia) of *Drosophila*. Mutation frequencies observed for doses ranging from 20 to 6000 R (all in acute treatments) do not show linear proportionality to dose, as would be the case when mature spermatozoa were irradiated.

To account for our data we have proposed a linear-quadratic model, which expresses the yield of mutations as $Y = C + \alpha D + \beta D^2$, where C is the computed control frequency, D is dose, and α and β are coefficients for one-track and two-track events. The values for C , α , and β are computed from the data by a weighted polynomial regression analysis (weighted by the inverse of the variance) and at this time are, respectively: 1.279×10^{-3} , 2.667×10^{-6} , 7.159×10^{-10} . This model suggests that dose-kinetics for recessive lethals is not essentially different from that for chromosome aberrations as previously thought. If this model is correct one would expect that equivalent high doses would produce different lethal frequencies depending on the manner of dose delivery, such as dose-rate and single dose (acute) treatment versus fractionation. This is currently being tested by our group for a total dose of 6000 R given in acute and fractionated treatments.

Unfortunately our research program has been limited since March of this year by the breakdown of the X-ray source in our Zoology Research Building which was almost always available. Since then we have continued our irradiations using a very similar X-ray machine located in the Department of Radiobiology; but this unit is in constant demand and we could only arrange to reserve it once a week on a regular basis. Thus we could continue to collect data for some acute high dose treatments, and for a fractionated series receiving 2 doses of 3000 R a week apart; we had already collected a fair number of data for the 4-fraction series (4 X 1500 %), but the planned experiments using 500 R fractions had to be postponed. At this writing funds for another X-ray machine for our Department have been pledged (in part by ERDA), a suitable machine has been found and should be installed soon.

EXPERIMENTAL PROCEDURES

The genetic scheme which was used for our previous investigations to detect newly arising sexlinked lethal mutations in females has remained unchanged. A condensed outline with diagram is again attached to this report.

The experimental P_1 females for the weekly irradiation experiments are collected as virgins, kept on sugar-agar medium at cool temperature and treated with various doses of 250 Kvp X-rays 16-25 hours after eclosion. As a rule each of the irradiation experiments consists of 3 treated groups and 3 corresponding, similarly processed control lots. The first

group of young females is exposed to an acute high dose (3000, 4500, or 6000 R) while a second group of young females receives the first installment of a fractionation regime; a third group, now one week older, is given its last installment at this time. This group had been irradiated first with the previous experiment, and in some cases had received additional dose fractions while being kept cool and on sugar-agar medium during the interval. Immediately after X-ray treatment is complete the treated P_1 females from groups 1 and 3, and those from corresponding untreated controls are mated in bottles to an excess of males. Every 2 or 3 days transfers to fresh culture medium are made to encourage egg proliferation. On day 15 after irradiation the males are removed and the females cultured individually in vials; by then F_1 emerging from these cultures should be derived from germ cells which were in gonial stage at time of treatment.

Cultures from P_1 -females carrying a pre-existing lethal in the experimental (y dow) chromosome can be recognized by inspection of the F_1 and are eliminated. 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 criteria 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) in relation 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.

RECENT RESULTS

Since the time of our last report (September, 1976) we have completed 47 more irradiation experiments, and tested over 61,000 X-chromosomes for new recessive lethal mutations. The recent data, from experiments 327 through 373, are as follows (lethal frequencies are not corrected for controls):

Treatment	No. Leth. / Tot. Tests	% Lethals
0 R	18/19,047	.095
Acute, 3000 R	77/ 4,579	1.682
Acute, 4500 R	358/12,501	2.864
Acute, 6000 R	189/ 4,734	3.992
2 fractions of 3000 R	439/11,801	3.720
4 fractions of 1500 R	274/ 8,352	3.281

Control data listed above are pooled from all "young" and "aged" lots processed at the same time as the treated groups. There is no detectable significant difference in spontaneous mutation frequency for only one week of aging.

We have so far accumulated some strictly comparable data for about 25,000 tests from each group; lethal frequencies and standard errors are: Young controls, $.096 \pm .020\%$; Aged controls, $.125 \pm .022\%$.

Delivery time for the higher doses was adjusted not to exceed the 20-minute time span during which chromosome breaks in *Drosophila* can still interact with each other. Dose rates used were 480 R/min with our own, and 280 R/min with the Radiology Department's X-ray machine. However, the fractionated treatment with 1500 R was given at the lower rate of 80 R/min, the same as previously used for acute doses of 1500 R.

The new data are not meaningful by themselves, but are consistent with previous results for 3000 and 6000 R; there were no previous acute exposure to 4500 R; the observed frequency for this new dose point is in good agreement with that predicted from our model, 2.778%.

The comparison of the effects of 6000 R, acutely given, and when fractionated needs more data. At this time, the yield of mutations is significantly different for four 1500 R fractions, but more data are needed to see whether it approximates the effect of the sum of the fractions.

DISCUSSION OF ACCUMULATED DATA

Table 1 represents an updated list of our data for sexlinked lethal frequencies from acute exposures to a wide range of doses. The most extensive data are for the low-dose range - up to 500 R. Lethal frequencies rise with dose (but

not in linear fashion) until they seem to reach a plateau for those 2 doses above 6000 R tested by us. The very high sterility of the P_1 -females at these high doses is probably due to gross chromosomal aberrations, which seem to selectively remove many severe mutants from the germ cell population.

Lethal rates in Table 1 include the spontaneous rates. Observed and computed values are in good agreement, with differences well within the standard error. Computed values were obtained from the equation which is shown in the table below the data.

Figure 1 is the graphic representation of the same data for doses up to 6000 R. Confidence limits shown for the observed data points are for one standard error. Two different regression lines are given; one is for a linear and the other for a linear-quadratic (parabolic) model of dose-kinetics. It is obvious that the latter shows the better fit.

Table 2 is a collection of data from dose fractionation experiments performed at various times during the course of our investigation. Data for 0, acute 6000 and for acute 1500 R are the same as in Table 1. The data for 2 x 3000 and 4 x 1500 R fractions are recent, the others not. P-values are based on the Chi-square test.

The upper part of the table gives a comparison of mutation frequencies obtained from acute exposures to 6000 R and from various fractionation regimes. The difference is in the expected direction; it is still non-significant for

the 2 x 3000 R group (more data are forthcoming) but highly significant when fractions of 2000 and 1500 R were used.

The lower portion of the table lists data for an acute 1500 R treatment compared to 3 x 500 R fractions. Both mutation frequencies are essentially the same. Experiments were discontinued since it would be impractical to search for an influence of dose fractionation using 1500 R. We have calculated now from the model equation that one-track (αD) and two-track (βD^2) events should contribute equally to the mutation frequency at a dose of approximately 3750 R. Therefore the search for the effect of dose fractionation now is made with higher total doses.

Since dose fractions are given two or more days apart we would expect no interaction between fractions, and the lethal frequencies from fractionation experiments should be equal to the sum of the effects of the fractions. At this time however, lethal rates for a total dose of 6000 R spread over a week's time in fractions of 3000, 2000 or 1500 R all seem higher than computed frequencies, which are respectively: 3.016%, 2.588% and 2.372%. More data might narrow this difference.

At this time it appears from our data that dose fractionation does indeed reduce X-ray induced mutational damage in oogonia, as expected from the linear-quadratic model. More precise numerical details have to be worked out in strictly controlled experiments, and it is essential to test a regime by which a total high dose is accumulated in low-dose (500 R) fractions.

BREEDING SCHEME

P₀: Balanced stock furnishing virgin females:

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

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

P₁ females X-rayed, mated en masse in bottles and transferred every 2 or 3 days to fresh culture medium until day 15 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:

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

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.

TABLE 1

SEX-LINKED LETHAL FREQUENCIES IN OOGONIA (ACUTE TREATMENTS)

Dose (R)	No. Replicate Experiments	Tot. No. Tests	Lethal Frequencies, %	
			Observed, $\pm$ S.E.	Computed*
0	368	163,213	.136 $\pm$.009	.128
20	147	67,553	.151 $\pm$.015	.133
100	224	102,491	.138 $\pm$.012	.155
200	164	92,685	.178 $\pm$.014	.184
500	317	118,169	.269 $\pm$.015	.279
1,500	76	45,534	.749 $\pm$.040	.689
3,000	43	21,602	1.537 $\pm$.084	1.572
4,500	23	12,501	2.864 $\pm$.149	2.778
6,000	17	8,880	4.178 $\pm$.212	4.305
7,500	5	1,962	4.074 $\pm$.435	6.155
9,000	7	1,082	4.529 $\pm$.632	8.327

* Model: $Y = 1.2794 \times 10^{-3} + 2.6672 \times 10^{-6} D + 7.1587 \times 10^{-10} D^2$, based on data for doses 0 to 6000R only.

FIGURE 1

TABLE 2

EFFECT OF DOSE FRACTIONATION ON THE INDUCTION OF SEX-LINKED LETHALS IN OOGONIA

No. X-ray Treatments, and Dose	No. Repli- cate Exp.	No. Leth. / Tot. Tests	% Lethals $\pm$ S.E.	P-Value, Acute vs. Fract.
0R*	368	222 / 162,991	.136 $\pm$.009	
1 x 6000R* (Acute)	17	371 / 8,880	4.178 $\pm$.212	--
2 x 3000R	23	424 / 11,221	3.779 $\pm$.180	.0929
3 x 2000R	10	209 / 7,387	2.829 $\pm$.193	<<.00001
4 x 1500R	17	274 / 8,352	3.281 $\pm$.195	.0193
1 x 1500R* (Acute)	76	341 / 45,193	.749 $\pm$.040	--
3 x 500R	20	59 / 8,467	.689 $\pm$.089	.518

* Entire data pooled

NEW PUBLICATION RELATED TO THIS PROJECT

Meyer, H.U. and S. Abrahamson. 1977. Linear-quadratic dose for X-ray induced recessive mutations in Drosophila oogonia - Abstract of paper presented at the second International Conference on Environmental Mutagens, Edinburgh, U.K., July 11-15, 1977.

STATEMENT OF TIME DEVOTED TO THIS PROJECT

The Principal Investigator, Seymour Abrahamson, has devoted 10% of his time during this 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 current period.