MASTER

GENETIC EFFECTS OF LOW X-RAY DOSES

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ABSTRACT

Large-scale experiments on the dose-kinetics of X-ray induced sex-linked lethal mutations in treated oogonia of Drosophila have generated the following data (lethal frequencies and standard errors in %, not corrected for control rate): 0R, 0.138 ± 0.011 (124,378 tests); 20R, 0.151 ± 0.015 (67,553); 100R, 0.138 ± 0.012 (102,491); 200R, 0.164 ± 0.015 (73,635); 500R, 0.270 ± 0.016 (99,902); 1500R, 0.778 ± 0.052 (28,677); 3000R, 1.392 ± 0.101 (13,502); 6000R (3 x 2000R), 2.829 ± 0.193 (7,387 tests). In these experiments clustering of identical mutations was avoided by testing only a single progeny from each parental female.

These 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 $C = 1.316 \times 10^{-3}$, $\alpha = 2.601 \times 10^{-6}$, $\beta = 6.378 \times 10^{-10}$, 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. We plan to

test the validity of this model in future experiments by comparing the mutation frequencies from equivalent high doses given in one acute treatment and in multiple fractions of a low dose.

INTRODUCTION

Though linear proportionality of radiation dose and induced frequencies of recessive mutations has been firmly established for mature germ cells of Drosophila, studies of low-dose kinetics for gonial cells have given conflicting evidence. While McSheehy's (1964) data for gamma-irradiated spermatogonia were compatible with linearity, Oftedal (1964) obtained a non-linear dose response in X-rayed spermatogonia giving a much greater per-roentgen yield of mutations with doses below 300R than with higher doses. His findings, if confirmed, would mean that our risk estimates for genetic radiation damage in the past have been too low, especially since immature germ cell stages persist for a long time during the life span of an organism and can accumulate mutations.

In view of the very low sensitivity of immature germ cells to mutation induction by ionizing radiations, a huge number of tests is necessary for the low dose points in such a study. Over the past seven years we have accumulated an extensive body of data, and now have made a new attempt to interpret these data.

EXPERIMENTAL AND GENETIC TECHNIQUES

Experimental procedures have remained the same during the entire course of our investigation. Mutability was measured by the frequency of newly arisen sex-linked lethal mutations. By testing oogonia instead of spermatogonia we avoided the loss of that portion of X-chromosome mutations which are subject to germinal selection.

The genetic scheme used throughout this investigation is summarized in Figure 1. The P_1 -females are collected as virgins and stored on minimal medium at 18°C to retard egg maturation until irradiation time, 16 to 24 hours after eclosion. Irradiations were always performed at 9 A.M.

3000 to 4000 P_1 -females were treated simultaneously with various X-ray doses in every experiment. Immediately after the treatment all females were mass-mated with an excess of males in bottles, and transformed to fresh culture medium several times. On day 13 after irradiation, all males are removed and the fertilized experimental P_1 -females cultured individually in vials with standard medium. The F_1 hatching from these cultures is therefore derived from eggs laid 13 days or more after irradiation, from cells in gonial stage at time of treatment.

Cultures of P_1 -females carrying a preexisting lethal in the experimental X-chromosome can be recognized by inspection of the F_1 -generations (absence of Y dow / y w B, FM6). Only a single F_1 -daughter from each experimental P_1 -female is tested for a possible new sex-linked

lethal. Thus clusters of identical mutations resulting from proliferation of an ancestral gonial cell are avoided.

All cases of suspected new lethals are routinely subjected to confirmation tests and classified on the basis of extensive progeny counts as to the degree of lethality, i.e. complete lethals, or partial lethals of various severity. A computer program allows us to obtain mutation frequencies for different definitions of a lethal. This makes it possible to relate our data to those of other investigators using other numerical criteria.

Though at the beginning our main interest was exploration of the low-dose effects (20-500R), it became necessary during the course of the investigations to also add some high dose points (1500, 3000 and 6000 R, (the last in 3 fractions of 2000R).

In all experiments contemporaneous treatments with several doses were performed as well as a control, though not all doses were included all the time. Doses investigated were 20, 100, 200, 500, 1500 and 3000 R, all given acutely and at the same dose rate (75-85 R/min). A G.E. Maxitron-300 Hospital-type X-ray machine was used at 250 Kvp. Our highest dose, 6000R, included in only 10 experiments, was not given as acute treatment, but over a week's time in three installments of 2000R. In all but 6 experiments, a contemporaneous 500R group was present, which makes this our common point of reference.

RECENT RESULTS

Since we submitted our last report (September 1974) we have completed 43 more radiation experiments, from which some 89,000 chromosomes were tested for newly arisen lethal mutations. As before, each of the tested X-chromosomes were derived from a different experimental parent-female.

The recent results, from experiments 229-278, are as follows (lethal frequencies not corrected for controls):

DOSE	NO. LETH./TOT. TESTS	%LETH.
0R	22 / 14,992	.1467%
100R	27 / 12,956	.2084%
200R	30 / 17,206	.1744%
500R	47 / 15,944	.2948%
1500R	176 / 23,129	.7609%
3000R	61 / 4,672	1.3057%

This new portion of our data, though not meaningful by itself, is in line with previous results, where 500R appeared to be a "doubling dose" for this biological system.

DISCUSSION OF ACCUMULATED DATA

A summary of the data obtained from 278 experiments completed so far is presented in Table 1, which lists lethal frequencies and standard errors for doses from 20R to 6000R with 95% confidence limits, based on the binomial distribution.

It is quite evident from the data that the individual mutation frequency is not linearly proportional to dose for the entire range of doses tested by us. In the past we had interpreted the fact that the mutation frequencies for 500R and lower doses were far less than expected, to be possibly due to repair of premutational damage, with such repair being inversely related to dose.

It now seems that 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 $C = 1.316 \times 10^{-3}$, $\alpha = 2.601 \times 10^{-6}$, $\beta = 6.378 \times 10^{-10}$, 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.

Half of the mutations recovered from 500R treated oogonia and the vast majority of those from lower doses must be of spontaneous origin. Since spontaneous mutation frequencies can fluctuate widely, we have compared the mutation frequencies of the low-dose treated groups with that of their contemporaneous controls. The pairwise comparisons are listed in Table 2, with the Chi-square significance (P) of the difference. This treatment of the data shows that the difference is significant for both 200 and 500 R, which is not the case when pooled data for all controls are contrasted with those from 200R. Needless to say that we plan to collect more data for this dose point, since the significant elevation of the lethal frequency above control level would support our conviction that there is no threshold effect at the low dose range.

Table 3 presents a comparison of observed sex-linked lethal frequencies and computed ones based on the linear-quadratic model. The Chi-square value for the data from 0 R to 3000 R is 12.5 (for 6 d.f.), indicative of the good fit of observed and computed frequencies.

Figure 2 depicts the theoretically expected dosage curve, with our observed values; also included are 2 dose points from the raw data published by Muller et al. (1963) for 4000R, given acutely and as chronic treatment. The acute dose results conform to expectation based on the quadratic model, while those from chronic exposures are in line with a linear increase in muta-

tion when the dose squared term becomes vanishingly small. Our empirically derived coefficients require that at a dose of 4000R approximately 50% of the induced mutation component was the result of dose² interaction kinetics, and therefore that this be the maximum reduction in mutation rate when the exposures at this dose were delivered chronically. Indeed our derived coefficients allow us to conclude that the experiments of Purdom (1963) which attempted to find dose-rate effects at doses in the range of 200-800 R in gonia cells were also destined to be negative since the dose² contribution over this dose range (in Drosophila) is 10% or less and therefore would be obscured by general statistical variability.

APPENDIX

The Principal Investigator (S.A.) has spent a major portion of the past year involved in analysis in all species and synthesis of mutation induction by high energy radiation. We feel that this effort (a considerable amount performed in collaboration with Professor Sheldon Wolff) is leading to a unifying model for eukaryotes that will have and indeed is having considerable bearing on interpretations of human genetic risk. We believe that fundamental radiobiological mechanisms are better understood when comparative radiation genetic synthesis is undertaken. As an example one of the very interesting contributions that is developing from this approach is that there is probably a phylogenetic relationship to target size diameters or site interaction distances for chromosome rearrangements. This subject will be dealt with in more detail in a subsequent publication, but suffice to say that what appeared to be inconsistencies in such derived interaction distances for different species, as with specific locus mutation studies, now appears to follow a similar phylogenetic pattern i.e. these site distances increase as the DNA content of the species increase. There will be apparent exceptions found to this statement but analysis of the conditions leading to these exceptions have already proven very illuminating and an understanding of the biology of the system now suggests why some exceptions will arise. As a case in point, studies by Abrahamson et al. (1971), and Parker and McCrone (1958) for the same germ cell stage gave (meiotic metaphase oocytes) different

site distance values for rearrangements. In Parker's experiments a Y-chromosome homologue was present which both paired and translocated with the X-chromosome being studied most frequently. In the Abrahamson experiment no Y-chromosome was present and the translocations most frequently involved autosomes with the X-chromosomes. This suggests to us that the average distance for interaction (exchange of broken ends) in metaphase was considerably greater in the Abrahamson experiments.

The major point we raise here is that when exception to expected relationships occur, one should not discard the rules but analyze the exceptional situations since they in turn should provide a deeper understanding of the conditions which apply. The chromosome theory of heredity was established because an exception in the form of non-disjunction was explored.

It appears that a general consistency is emerging from genetic studies (mutation and rearrangements), cell survival studies and possibly somatic effects (tumor induction and life shortening) in mammals, all these endpoints appear to follow a quadratic induction relationship. The average target diameter size is in the range of 1 micron, suggesting that the initiating event in each case is chromosome breakage.

Another suggestion that emerges from this form of analysis is that the Tradescantia "mutation" scoring system of Sparrow's may well be one of the most efficient for the testing of environmental mutagens because the system provides a larger target size than most systems presently used and therefore the exquisite

sensitivity demonstrated by Sparrow for X-ray and neutron induction should be profitably employed for detection of other genetic hazards. The major issue here is that again the comparative approach will highlight the most efficient systems that may be employed in assessing genetic hazards and thus lead to a better method of extrapolating these hazards to man.

FREQUENCIES OF SEXLINKED LETHALS, ALL DATA

Dose (R)	No. Lethals		% Leth. $\pm$ s.e.	95% Confid. Intervals*
	No.	Tot. Tests		
0	174	124,378	.1383 $\pm$.0105	(.1162, .1633)
20	102	67,553	.1510 $\pm$.0149	(.1231, .1833)
100	141	102,491	.1376 $\pm$.0116	(.1156, .1627)
200	121	73,635	.1643 $\pm$.0149	(.1364, .1963)
500	270	99,902	.2703 $\pm$.0164	(.2390, .3044)
1500	223	28,677	.7776 $\pm$.0519	(.6792, .8862)
3000	188	13,502	1.3924 $\pm$.1008	(1.2016, 1.6046)
6000**	209	7,387	2.8293 $\pm$.1929	(2.4631, 3.2329)

* Based on binomial distribution

**3 fractions of 2000R

TABLE I

SEX-LINKED LETHAL FREQUENCIES OF LOW-DOSE IRRADIATED
GROUPS COMPARED WITH THEIR CONTEMPORANEOUS CONTROL
GROUP, AND CHI-SQUARE SIGNIFICANCE (P).

Comparison:		No. Lethals			Chi-Square
No. Exp.	Dose (R)	Total Tests	% Leth. ± s.e.		Significance (P)
147	20	102/67,553	.1510 ± .0149		.9175
	0	112/73,132	.1531 ± .0145		
247	100	141/102,491	.1376 ± .0116		.5044
	0	135/106,336	.1270 ± .0109		
138	200	121/73,635	.1643 ± .0149		.0150
	0	68/59,682	.1139 ± .0138		
272	500	270/99,902	.2703 ± .0164		.0000
	0	172/124,378	.1383 ± .0105		

TABLE 2

COMPARISON OF OBSERVED AND COMPUTED FREQUENCIES
OF SEX-LINKED LETHAL MUTATIONS
(DATA POOLED FROM 278 EXPERIMENTS)

X-RAY DOSE	MUTATION FREQUENCIES	
	OBSERVED	COMPUTED *
0R	1.383×10^{-3}	1.317×10^{-3}
20R	1.510×10^{-3}	1.369×10^{-3}
100R	1.377×10^{-3}	1.583×10^{-3}
200R	1.643×10^{-3}	1.862×10^{-3}
500R	2.703×10^{-3}	2.776×10^{-3}
1500R	7.776×10^{-3}	6.653×10^{-3}
3000R	1.392×10^{-2}	1.486×10^{-2}
3 x 2000R	2.829×10^{-2}	2.458×10^{-2}

* Based on model: $Y = C + \alpha D + \beta D^2$, where $C = 1.317 \times 10^{-3}$,
 $\alpha = 2.601 \times 10^{-6}$, $\beta = 6.378 \times 10^{-10}$

TABLE 3

P₀: Balanced stock furnishing virgin females:

$$\frac{y \quad \text{dow}^*}{\text{Df}(1^{J1} \quad y \quad \text{ac}), \text{sc}^{Sl} \quad B \quad \text{InS} \quad w^a \quad \text{sc}^8} \times \frac{\text{Df, Basc}}{y+\text{sc}^8 \cdot Y} \delta\delta$$

"Df, Basc"

P₁:

Virgin ♀♀	$\frac{y \quad \text{dow}}{\text{Df, Basc}}$	x	$\frac{y^{3ld} \quad w^{FM6} \quad B, \text{InsFM6}}{y^+ \quad (\text{normal } Y)}$	♂♂
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P₁ females X-rayed, mated en masse in bottles, brooded several times until day 13 after irradiation. Males removed, and fertilized females bred individually.

F₁: Each culture is inspected for presence of a reasonable proportion of y dow males to exclude preexisting lethals. If satisfactory:

One single F ₁ daughter	$\frac{y \quad \text{dow}}{y^{3ld} \quad w^{FM6} \quad B, FM6}$	x	$\frac{y^{3ld} \quad w^{FM6} \quad B, FM6}{y^+}$	♂♂
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"automatically virgin" (*)

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

F₃: Confirmation crosses.

(*) 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.

Fig. 1. Genetic Scheme for Detection of Sex-Linked Lethal Mutations in Females.

MUTATION RESPONSE IN OOGONIA

FIGURE 2

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OTHER FINANCIAL ASSISTANCE

Because of the reduction of our proposed budget by the ERDA ~~Atomic Energy Agency~~ for the current grant period, we had to seek additional financial assistance, since this type of experiment requires a large-scale group effort. We were fortunate to obtain additional support, through a NIH grant to the Department of Genetics, in the amount of \$34,687 (direct cost only) for the period from August 1, 1975 through July 31, 1976 - a period which overlaps that of this contract.

However, we are told that the Genetics Project Program Grant from the NIH is going to be phased out and that such additional support for our project might not be available during the next year. We hope that it will be possible to get our entire support again through the U.S. Energy Research and Development Administration.